Part I: Total Synthesis of Variecolortides A–C

Part II: Towards the Total Synthesis of Naphthomycin K and Divergolides C and D

Part III: Synthesis of Photochromic Open-Channel Blockers

von

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aus

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Erklärung

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Eidesstattliche Versicherung

Diese Dissertation wurde eigenständig und ohne unerlaubte Hilfe erarbeitet.

München, ...

.....
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Dedicated to Christin and my family.

„Es ist leichter zum Mars vorzudringen, als zu sich selbst.“
(C. G. Jung)

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Publications, Conference Contributions and Awards Resulting from this Thesis

Publications

Peer-Reviewed

1) **Kuttruff, C. A.**; Mayer, P.; Trauner, D., Evolution of a Synthetic Strategy for the Variecolortides, *Eur. J. Org. Chem*. **2012**, Article *ASAP*; DOI: 10.1002/ejoc.201200712.

2) Fehrentz, T.[1]; **Kuttruff, C. A.**[1]; Huber, F. M. E.; Kienzler, M. A.; Mayer, P.; Trauner, D., Exploring the Pharmacology and Action-Spectra of Photochromic Open-Channel Blockers, *ChemBioChem* **2012**, *13*, 1746–1749; DOI: 10.1002/cbic.201200216.

3) **Kuttruff, C. A.**; Geiger, S.; Cakmak, M.; Mayer, P.; Trauner, D., An Approach to Aminonaphthoquinone Ansamycins Using a Modified Danishefsky Diene, *Org. Lett.* **2012**, *14*, 1070–1073; DOI: 10.1021/ol203437a.

4) **Kuttruff, C. A.**; Zipse, H.; Trauner, D., Concise Total Syntheses of Variecolortides A and B through an Unusual Hetero-Diels-Alder Reaction, *Angew. Chem. Int. Ed.* **2011**, *50*, 1402–1405; DOI: 10.1002/anie.201006154. *Highlighted in Synfacts 2011, 577–694.*

   Kuttruff, **C. A.**; Zipse, H.; Trauner, D., Kompakte Totalsynthese von Variecolortid A und B durch eine ungewöhnliche Hetero-Diels-Alder Reaktion, *Angew. Chem.* **2011**, *123*, 1438–1441; DOI: 10.1002/anie.201006154.

Conference Contributions

- **Gordon Research Conference on Natural Products, Andover (NH), USA (07/2012)**

  Poster: C. A. Kuttruff, H. Zipse, D. Trauner, Total Synthesis of Variecolortides A–C.

- **14th Asian Chemical Congress, Bangkok, Thailand (09/2011)**

  Poster: C. A. Kuttruff, H. Zipse, D. Trauner, Concise Total Syntheses of Variecolortides A, B and C.

- **Pacifichem 2010, Honolulu, Hawaii, USA (12/2010)**

  Poster: C. A. Kuttruff, H. Zipse, D. Trauner, Concise Total Synthesis of Variecolortides A, B and C.

[1] These authors contributed equally to this work.

- **CIPSM-Wildbad-Kreuth-Konferenz, Kreuth, Deutschland (09/2010)**

  Poster: C. A. Kuttruff, H. Zipse, D. Trauner, Concise Total Synthesis of Variecolortide A and B.

- **Bayer Ph.D. Student Course 2009, Monheim, Deutschland (07/2009)**

  Poster: C. A. Kuttruff, M. Cakmak, D. Trauner, Towards the Total Synthesis of Naphthomycin K.

Awards

- 06/2012 **Finalist of the DSM Science & Technology Awards 2012**
- 06/2011 **Finalist of the 2011 Reaxys PhD Prize**

Abstract

Part I: Total Synthesis of Variecolortides A–C

The variecolortides are structurally intriguing, fungal natural products that present a unique merger of three major streams of biosynthesis. They can be traced back to other known natural products, namely the viocristins and echinulins, and we wanted to prove if they could somehow come together to form the variecolortides. Thus, these natural products were prepared in short sequences and their critical linkage was subsequently investigated. It was found that an unprecedented hetero-Diels-Alder reaction effected this linkage and furnished the central spirocyclic core of the variecolortides. Ultimately, a highly convergent route to all three variecolortides (A–C) was developed. The natural products were each efficiently synthesized as racemates in seven steps or less (longest linear sequence), largely avoiding protecting group chemistry.

Part II: Towards the Total Synthesis of Naphthomycin K and Divergolides C and D

Ansamycins are an important class of natural products that often show potent antibacterial and antiviral activities. We have engaged in the total syntheses of several new aminonaphthoquinone ansamycins, including naphthomycin K and divergolides C and D. A unified approach to their naphthoquinone core was targeted and led to the development of a novel, cyano-substituted Danishefsky-type diene. The latter was used in the synthesis of cyano-substituted naphthoquinones, naphthalenes and other highly functionalized small molecules. Since the cyano-substituted naphthoquinones could not be further elaborated into the desired natural products, alternatively substituted bromonaphthalenes were instead synthesized over short sequences. In addition, several strategies towards the synthesis of an *ansa* chain fragment of divergolides C and D were developed.

Part III: Synthesis of Photochromic Open-Channel Blockers

Optical control of transmembrane ion channel function with high temporal and spatial precision opens the door for a better understanding of neuronal circuits and pharmacological associated questions. Several red-shifted derivatives of QAQ (quaternary ammonium-azobenzene-quaternary ammonium), a powerful doubly charged photochromic blocker, have been synthesized. They allow for remote control of K_v and Na_v channel conductance with light and offer the opportunity to reversibly silence neuronal activity.

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Part I: Total Synthesis of Variecolortides A–C

1 Introduction

Nature remains a major source of inspiration for synthetic chemists, not only in terms of the structures it produces, but also with respect to the strategies it uses for their construction. These have been mimicked with remarkable success in the laboratory, yielding many elegant and efficient syntheses of natural products.[1] Many of these "biomimetic" syntheses incorporate reaction cascades, the role of which in the origin of natural products has been increasingly recognized.[2] Cascade reactions can create several stereocenters in a single step, which allows for the spontaneous construction of very complex chiral molecules from achiral precursors. In the absence of an asymmetric environment (e.g. an enzyme pocket) however, there is no reason why one enantiomer should be preferred over the other. Hence, complex natural products, that have been isolated in racemic form (Figure 1) can be suspected to arise through cycloadditions or cascade reactions in a more or less spontaneous fashion and thus are prime candidates for biomimetic total synthesis.

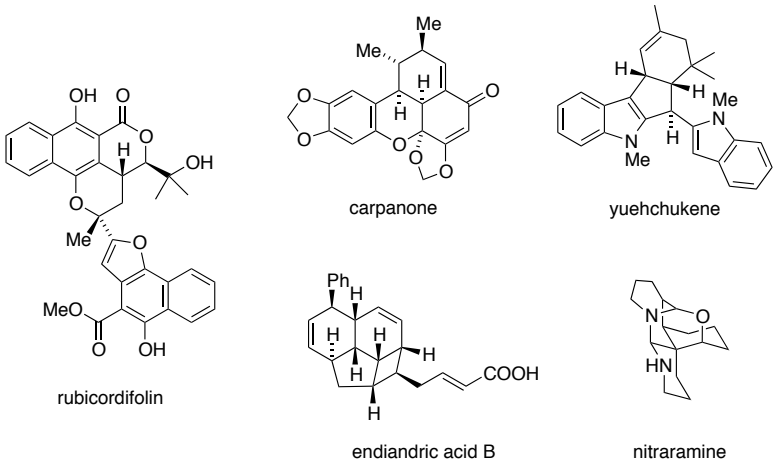


Figure 1. A selection of racemic natural products that have been made through biomimetic cascade reactions.

1.1 Isolation and Structure

Shortly after we had published our biomimetic synthesis of the potent indolamine-2,3-dioxygenase (IDO) inhibitors exiguamines A (**1.1**) and B (**1.2**),[3] the variecolortides A–C (**1.3**–

[1] Razzak, M.; De Brabander, J. K. *Nat. Chem. Biol.* **2011**, *7*, 865–875.
[2] Nicolaou, K. C.; Edmonds, D. J.; Bulger, P. G. *Angew. Chem. Int. Ed.* **2006**, *45*, 7134–7186.
[3] Volgraf, M.; Lumb, J.-P.; Brastianos, H. C.; Carr, G.; Chung, M. K. W.; Münzel, M.; Mauk, A. G.; Andersen, R. J.; Trauner, D. *Nat. Chem. Biol.* **2008**, *4*, 535–537.

1.5) attracted our attention. These alkaloids, which occur as racemates in nature, were isolated from the halotolerant fungal strain *Aspergillus variecolor* B-17 and their structures and biological activities were reported by Wang *et al.* in 2007.[4] We were particularly captivated by the intriguing structure of the variecolortides. They each feature a pyrano-anthrone moiety which contains four conjugated rings and is connected to a central diketopiperazine moiety that forms a central spirocyclic *N,O*-acetal. A similar structural motif can also be found in the exiguamines. The central diketopiperazine moiety is further connected to a reversely prenylated indole, which additionally possesses either a hydrogen or a prenyl group at C22.

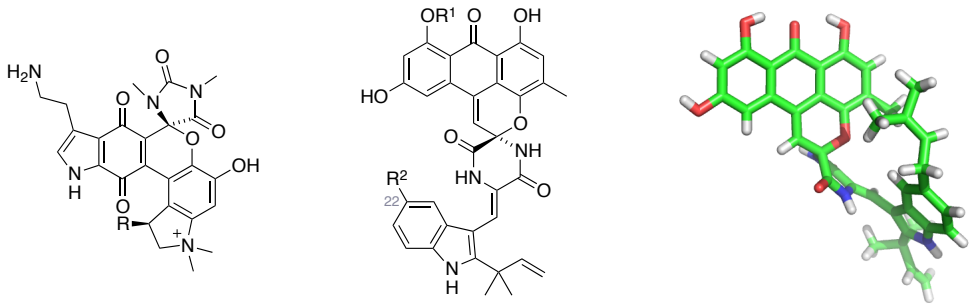


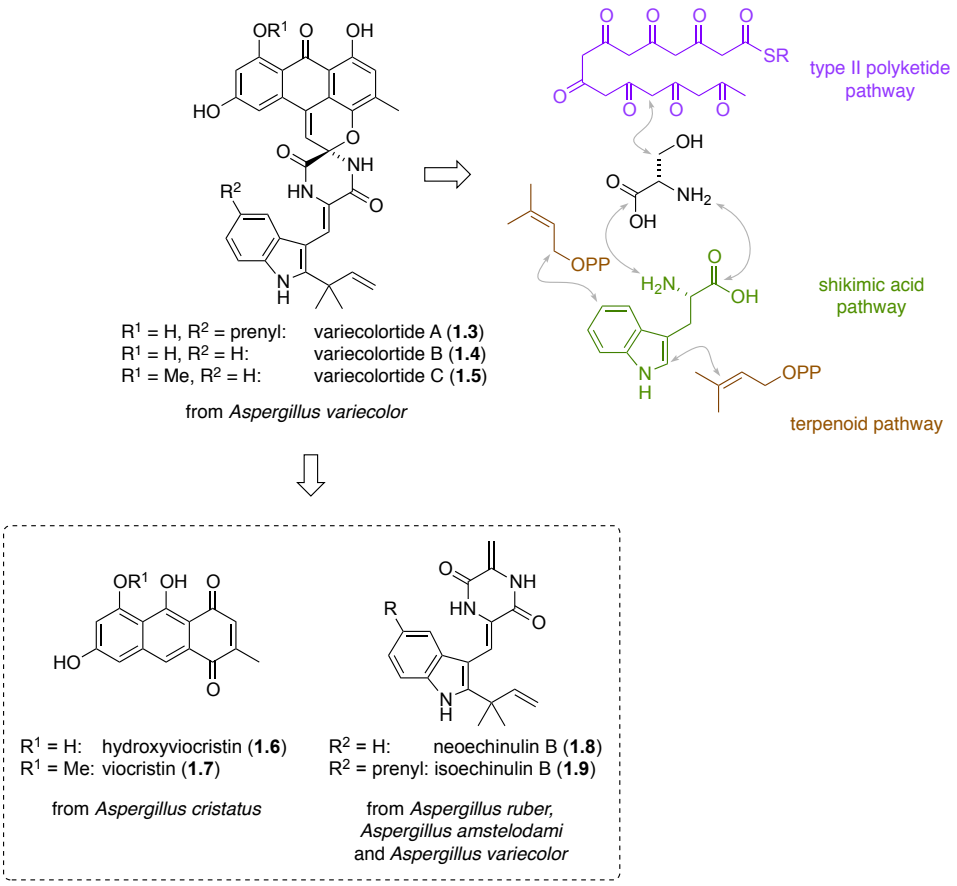
Figure 2. Structures of exiguamines A (**1.1**) and B (**1.2**) and variecolortides A–C (**1.3**–**1.5**).

In terms of their biological activity, the variecolortides have been found to display cytotoxic activity against K-562 human leukemia cells and weak radical scavenging activity against the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical. However, further investigations are necessary to explore the pharmacological relevance of these fungal natural products.

1.2 Proposed Biosynthesis

Not only did we find their unique connectivity particularly appealing, but we were also drawn to the fact that each of the substructures present in the variecolortides stem from a different biosynthetic pathway. As such, these alkaloids present an intriguing union of three different biosynthesis pathways: the type II polyketide pathway for the northern anthraquinone moiety, the shikimic acid pathway for the aromatic amino acid tryptophan and the terpenoid pathway for the prenyl- and reverse-prenyl side chains (Scheme 1). To the best of our knowledge, this is the first example of such a combination of biosynthetic pathways reported to date.

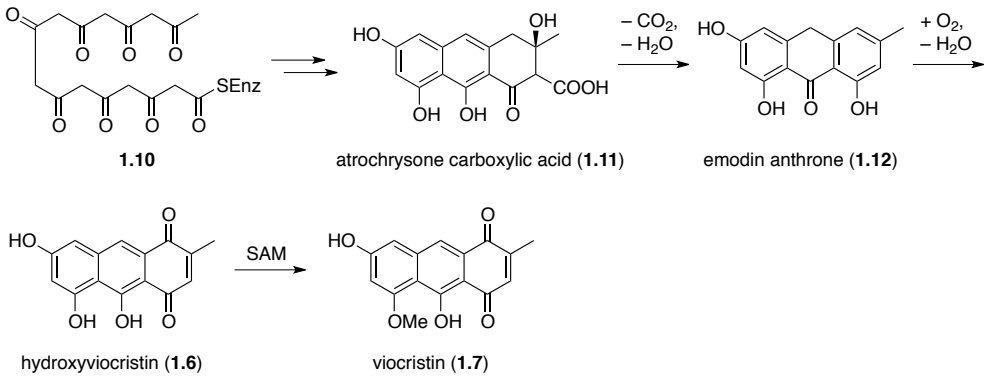
[4] Wang, W.-L.; Zhu, T.-J.; Tao, H.-W.; Lu, Z.-Y.; Fang, Y.-C.; Gu, Q.-Q.; Zhu, W.-M. *Chem. Biodiversity* **2007**, *4*, 2913–2919.



Scheme 1. Biosynthetic analysis of the variecolortides.

A retro-biosynthetic analysis of the variecolortides suggests that they may derive from known, albeit less complex natural products (Scheme 1). Specifically, we think that the northern part of the variecolortides can be traced back to hydroxyviocristin (**1.6**) and viocristin (**1.7**) respectively. These naturally occurring 1,4-anthraquinones have been isolated from *Aspergillus cristatus* by Laatsch and coworkers in 1982.[5] Their postulated biosynthesis follows the well-studied polyketide pathway.[6] As depicted in Scheme 2, octaketide **1.10**, derived from acetyl- and malonyl-CoA, would be transformed into atrochrysone carboxylic acid (**1.11**) through a series of steps involving aldol reactions, dehydration, enolization and hydrolysis from the enzyme. Decarboxylation and subsequent dehydration of **1.11** would lead to emodin anthrone (**1.12**), which upon oxidation and tautomerization forms hydroxyviocristin (**1.6**). Selective methylation by *S*-Adenosylmethionine (SAM) finally would afford viocristin (**1.7**).

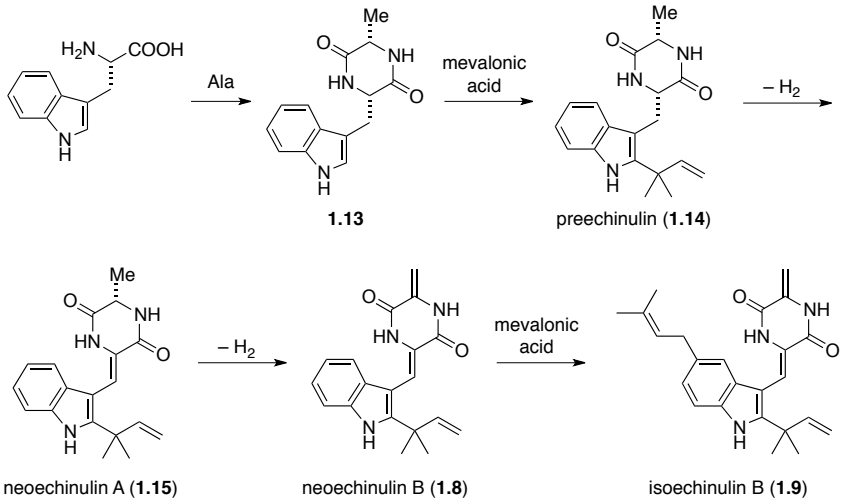
[5] Laatsch, H.; Anke, H. *Liebigs Ann. Chem.* **1982**, 2189–2215.
[6] Dewick, P. M. *Medicinal Natural Products: A Biosynthetic Approach*, 3rd ed.; John Wiley & Sons Ltd: Chichester, 2009.



Scheme 2. Proposed polyketide pathway for the biosynthesis of hydroxyviocristin (**1.6**) and viocristin (**1.7**).

The southern part of the variecolortides may arise from neoechinulin B (**1.8**)[7] and isoechinulin B (**1.9**)[8]. These natural products were isolated independently from each other in the 1970s from the mycelia of *Aspergillus ruber* and *Aspergillus amstelodami* respectively. In 2007, Wang *et al.* reported their reisolation together with several other analogues from the broth of the halotolerant fungus *Aspergillus variecolor*.[9] In addition, Wang and coworkers also proposed a mixed amino acid-mevalonic acid pathway for the biosynthesis of these alkaloids (depicted in Scheme 3 for neoechinulin B (**1.8**) and isoechinulin B (**1.9**)). Starting from the amino acids tryptophan and alanine, cyclo(Trp-Ala) (**1.13**) would be formed in the first step by intermolecular cyclization. Further reaction with mevalonic acid would give preechinulin (**1.14**).[10], which in turn could be dehydrogenated to give neoechinulin A (**1.15**). Proceeding from this natural product, one more dehydrogenation step would lead to the formation of neoechinulin B (**1.8**), which could be reversely-prenylated by reaction with mevalonic acid to form isoechinulin B (**1.9**).

[7] (a) Dossena, A.; Marchelli, R.; Pochini, A. *J. Chem. Soc., Chem. Comm.* **1974**, 771–772; (b) Marchelli, R.; Dossena, A.; Pochini, A.; Dradi, E. *J. Chem. Soc., Perkin Trans. 1*, **1977**, 713–717.
[8] Nagasawa, H.; Isogai, A.; Suzuki, A.; Tamura, S. *Tetrahedron Lett.* **1976**, *19*, 1601–1604.
[9] Wang, W.-L.; Lu, Z.-Y.; Tao, H.-W.; Zhu, T.-J.; Fang, Y.-C.; Gu, Q.-Q.; Zhu, W.-M. *J. Nat. Prod.* **2007**, *70*, 1558–1564.
[10] Hamasaki, T.; Nagayama, K.; Hatsuda, Y. *Agric. Biol. Chem.* **1976**, *40*, 203–205.



Scheme 3. Postulated biosynthetic pathway to neoechinulin B (**1.8**) and isoechinulin B (**1.9**).

We were fascinated by the question, if these natural products could indeed come together to form the variecolortides, prompting us to explore their biomimetic synthesis.

2 Results

2.1 Published Results

2.1.1 Concise Total Syntheses of Variecolortides A and B through an Unusual Hetero-Diels-Alder Reaction

Publication: Kuttruff, C. A.; Zipse, H.; Trauner, D. *Angew. Chem. Int. Ed.* **2011**, *50*, 1402–1405 and Kuttruff, C. A.; Zipse, H.; Trauner, D. *Angew. Chem.* **2011**, *123*, 1438–1441.

Communications

Natural Product Synthesis

DOI: 10.1002/anie.201006154

Concise Total Syntheses of Variecolortides A and B through an Unusual Hetero-Diels–Alder Reaction**

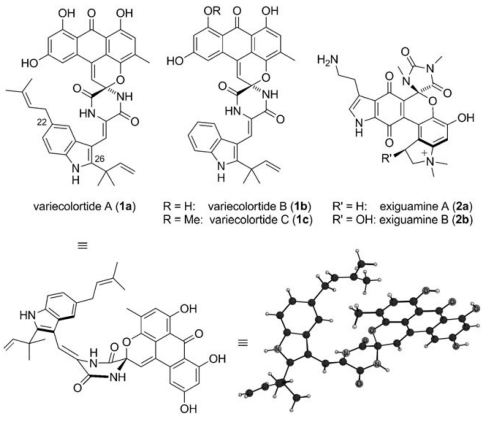
Christian A. Kuttruff, Hendrik Zipse, and Dirk Trauner**

Speculations on the biosynthetic origin of natural products continue to inspire synthetic chemists and have led to many elegant and efficient total syntheses.[1] Many of these involve cascade reactions, where a high-energy substrate is formed and then spontaneously reacts further to create increasingly more complex products.[2] As such, biomimetic strategies do not rely on the use of sophisticated enzymes, which are usually, but not always, required in a "real" biosynthesis.

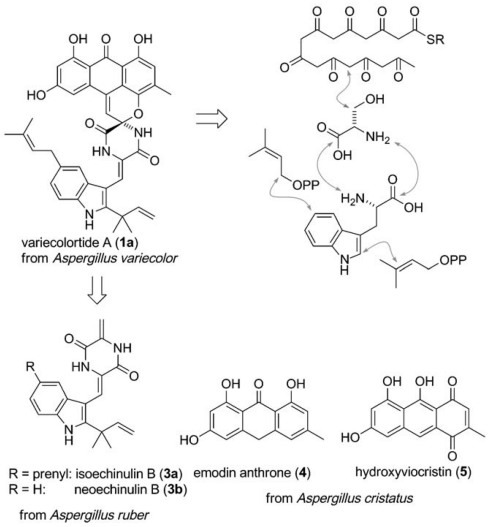
We recently published a synthesis of the dopamine-derived alkaloids exiguamine A and B (**2a, b**) that relied on a biomimetic pericyclic reaction and oxidation cascade (Scheme 1).[3] The exiguamines occur as racemates in nature and feature a spirobicyclic N,O-acetal as a key structural element. It was therefore unsurprising that the variecolortides (**1a–c**), a newly disclosed family of racemic N,O-acetals, piqued our interest.[4] These unusual fungal natural products were isolated from a halotolerant strain of the fungus *Aspergillus variecolor* and were shown to have modest cytotoxic effects.[4]

Structurally, the variecolortides feature an unprecedented 9,10-anthraquinone methide moiety fused to a dihydropyran (Scheme 1). This flat tetracyclic ring system is further linked to a diketopiperazine, generating a spirocyclic N,O-acetal. The central diketopiperazine is also coupled to an indole, which is reverse-prenylated at C26. Variecolortide A (**1a**) is the most complex member of the family, owing to the additional prenyl group at C22 of the indole nucleus, which is lacking in variecolortide B (**1b**) and its methyl ether variecolortide C (**1c**).

Biosynthetically, the variecolortides appear to stem from a C_{16} polyketide, the amino acids serine and tryptophan, and one or two equivalents of dimethylallyl pyrophosphate (Scheme 2). As such, they represent a unique merger of three major streams of biosynthesis: the shikimic acid pathway (for the aromatic amino acid tryptophan), the type II polyketide pathway (for the anthraquinone derivative), and



Scheme 1. Racemic natural products featuring N,O-acetals.



Scheme 2. Biosynthetic analysis of the variecolortides. PP = diphosphate.

[*] C. A. Kuttruff, Prof. Dr. H. Zipse, Prof. D. Trauner
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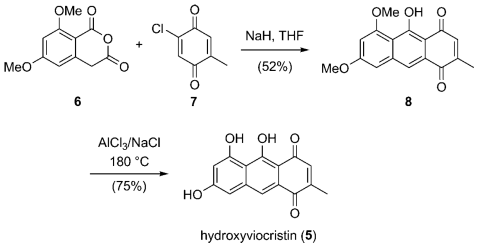
the terpenoid pathway (for the prenyl and reverse-prenyl side chains). While many natural compounds are known that integrate three or more different biosynthetic pathways, this particular combination appears to be rare if not unknown.

Natural products that exhibit a subset of the structural features of the variecolortides, however, are well known. Several decades ago, Laatsch and Anke reported the structures of emodin anthrone (**4**) and hydroxyviocristin (**5**), an anthrone and a 1,4-anthraquinone, respectively, that bear a substitution pattern corresponding to the variecolortides (Scheme 2).[5] These compounds were isolated from *Aspergillus cristatus*. The unsaturated diketopiperazine isoechinulin B (**3a**)[6] was obtained from *Aspergillus ruber*, along with several congeners that lack a prenyl moiety or in which the *exo*-methylene moiety is oxidatively cleaved, hydrogenated, or masked as a methanol adduct.[7] At the outset of our studies it was not clear, however, how these natural products, which have not been reported to occur in *Aspergillus variecolor*, would come together to form the variecolortides. We now report a concise total synthesis of **1a** and **1b** that offers a surprisingly simple solution for the linkage of the anthraquinone and diketopiperazine components and incorporates a new type of Diels–Alder reaction[8] that could be biosynthetically relevant.

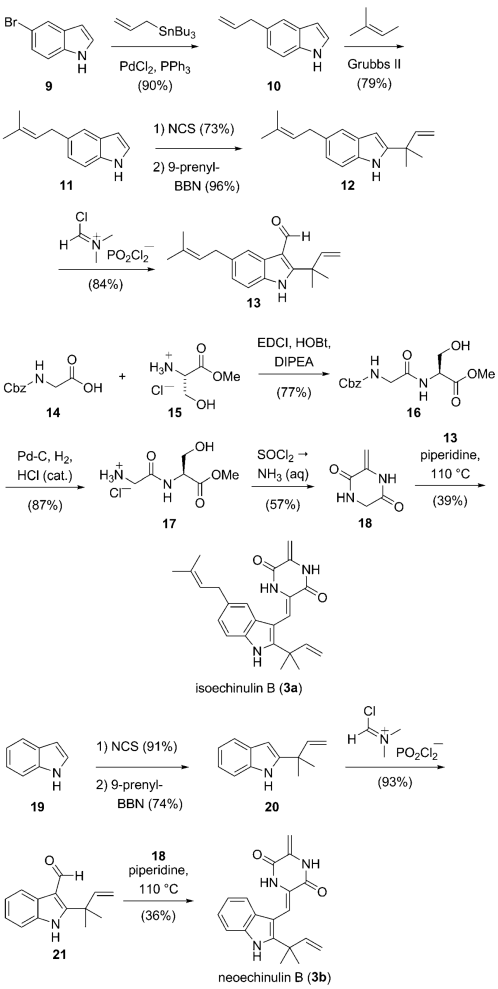
Our total synthesis of the variecolortides started with the preparation of hydroxyviocristin (**5**; Scheme 3). Deprotonation of the known orsellinic acid derived anhydride **6**,[9]



Scheme 3. Total synthesis of hydroxyviocristin (**5**).

followed by addition of the resultant benzylic anion to chloro *para*-benzoquinone **7** resulted in a conjugate addition/decarboxylation sequence to give the known 1,4-anthraquinone **8**.[10] Subsequent demethylation of this material in molten $AlCl_3$/NaCl yielded hydroxyviocristin (**5**).

The synthesis of the diketopiperazine–indole component started with palladium-catalyzed cross-coupling of 5-bromoindole (**9**) with tributylallylstannane, followed by Grubbs olefin cross-metathesis to give prenylated indole **11** (Scheme 4). Installation of the remaining prenyl group in the reverse sense was achieved by subjecting **11** to Danishefsky conditions to give **12**.[11] Intermediate **12** was then formylated with the Vilsmeier reagent to give **13**. In order to incorporate the amino acid portion, carbobenzoxy-protected glycine **14** was condensed with serine methyl ester (**15**) to afford dipeptide **16**, which after removal of the Cbz group, underwent cyclization and formal elimination of water to



Scheme 4. Total synthesis of isoechinulin B (**3a**) and neoechinulin B (**3b**). BBN = 9-borabicyclo[3.3.1]nonane, Cbz = benzyloxycarbonyl, DIPEA = *N*,*N*-diisopropylethylamine, EDCI = *N*′-(3-dimethylaminopropyl)-*N*-ethylcarbodiimide, HOBt = 1-hydroxybenzotriazole, NCS = *N*-chlorosuccinimide.

afford *exo*-methylene diketopiperazine **18**. Following a protocol developed by Kishi et al., we condensed this material with formyl indole **13** to afford isoechinulin B (**3a**) as a single isomer.[12] An analogous sequence starting from indole (**19**→**20**→**21**) provided neoechinulin B (**3b**; Scheme 4).

With both hydroxyviocristin (**5**) and the echinulins (**3a, 3b**) in hand, we were in a position to investigate the critical coupling of these components. Our original plan had called for the use of nucleophilic or radical additions using either **5** or emodin anthrone (**4**) as the polyketide building block.

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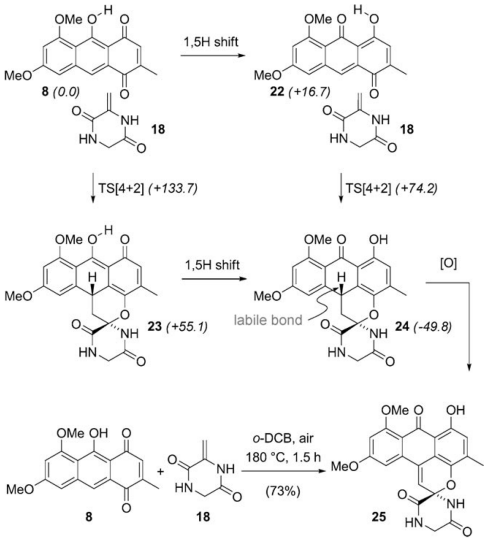
However, this strategy was eventually abandoned because of the poor reactivity of *exo*-methylene diketopiperazines towards anionic nucleophiles and our inability to control the radical additions of emodin anthrone. We therefore turned our attention to a cycloaddition strategy, which was initially explored experimentally and computationally with a model system (Scheme 5). We reasoned that hydroxyviocristin (**5**)



Scheme 5. A model system for the key cycloaddition. The relative energies of key intermediates and activation barriers are indicated in brackets (see text). *o*-DCB = *ortho*-dichlorobenzene, TS[4+2] = transition state of the [4+2] cycloaddition.

and its dimethyl ether **8** could undergo an intramolecular 1,5-hydrogen shift to afford the quinone methide tautomer **22**. This reactive intermediate would be prone to undergo a hetero-Diels–Alder cycloaddition with *exo*-methylene diketopiperazine **18** to afford spiro-N,O-acetal **24**. Being an anthrone, such an intermediate exhibits an extremely labile benzylic C–H bond and would undergo rapid oxidation in the presence of air to yield the 9,10-anthraquinone methide **25**. Indeed, when **8** and **18** were heated together in a sealed tube with an aerobic headspace, **25** was isolated as the only identifiable product (Scheme 5). This compound corresponds to the upper portion of the variecolortides and could even be an intermediate in their synthesis.

Our proposed Diels–Alder/oxidation mechanism is supported by density functional calculations performed at B3LYP/6-31G(d) level (see Scheme 5 and Figure S1 in the Supporting Information). According to these calculations, tautomer **8** reacts with **18** through a concerted, asynchronous pathway to yield cycloaddition product **23**. This process is considerably endothermic (by 55.1 kJ mol^{-1}) and faces a large reaction barrier of + 133.7 kJ mol^{-1}. By contrast, tautomer **22** is slightly less stable than **8** by 16.7 kJ mol^{-1}, but significantly more reactive with respect to the Diels–Alder reaction with dienophile **18**. The reaction barrier now amounts to a mere + 74.2 kJ mol^{-1}, and formation of the cycloaddition product **24** is exothermic by 49.8 kJ mol^{-1}. Owing to the considerable differences in reaction energetics and reaction barriers, it is clear that only cycloaddition through tautomer **22** is relevant under the experimental conditions.

The calculated geometry of the transition state of this reaction is depicted in Figure 1. Our calculations also show that the *exo*-methylene diketopiperazine **18** functions as the nucleophilic component in an asynchronous cycloaddition, wherein the carbon–carbon bond is formed to a larger extent than the carbon–oxygen bond in the transition state.

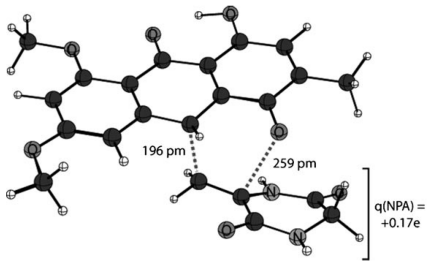
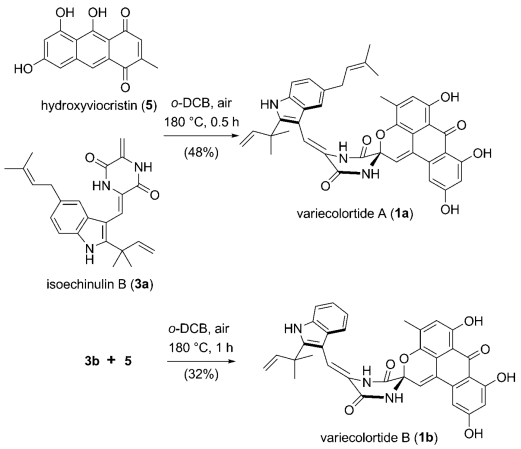


Figure 1. Calculated transition state of the asynchronous hetero-Diels–Alder reaction involving **22** and **18**. The lengths of the forming bonds and the partial charge *q*(NPA) of the heterodienophile are indicated. NPA = natural population analysis.

In order to assess the feasibility of the proposed oxidation of **24** to give the isolated reaction product **25**, the stability of the bisbenzylic C–H bond indicated in Scheme 5 was calculated using a series of reference compounds at the G3B3 level of theory. Using appropriate isodesmic reactions the C–H bond dissociation energy (BDE) for this C–H bond amounts to + 315.9 kJ mol^{-1} (see the Supporting Information for details). This value is even less than that found in common reducing agents such as 1,4-cyclohexadiene [BDE(C–H) = + 318.0 kJ mol^{-1})], $HSnBu_3$ [BDE(Sn–H) = + 328.9 kJ mol^{-1}], and thiophenol [BDE(S–H) = + 335.4 kJ mol^{-1}] and thus in full support of the in situ oxidation pathway proposed above.[13]

Armed with these insights and having optimized our key step with model compounds, we proceeded to complete the syntheses of the variecolortides (Scheme 6). We anticipated that the disubstituted *exo*-methylene moiety in the echinulins would be the most reactive heterodienophile and that the correct regioisomers would be formed. We were pleased to find that heating of hydroxyviocristin (**5**) and isoechinulin B (**3a**) in *ortho*-dichlorobenzene indeed afforded variecolortide A in 48% yield. Similarly, variecolortide B was obtained from building blocks **3b** and **5**. The modest yields of these key reactions probably reflect the known instability of hydroxyviocristin.[5] The variecolortides were the only isomers isolated under these conditions; no regioisomers and no



Scheme 6. The total synthesis of variecolortide A and B.

reaction with other double bonds present in the echinulins were observed.

While the conditions employed in our key reaction can certainly not be deemed biomimetic, the concerted nature of the reaction does raise some interesting biosynthetic questions. Preliminary experiments under more biological conditions (aqueous phosphate buffer at ambient temperature) have failed to yield any identifiable products. Given these results, and the calculated activation barriers, it appears that the variecolortides are not the products of an adventitious, uncatalyzed Diels–Alder reaction occurring in the fungus. This raises the interesting possibility that a "Diels–Alderase" is involved.[14] Also, while it is entirely conceivable that **5** and **3a**/**3b** are the true biosynthetic precursors of the variecolortides, the possibility that the linkage takes place before the polyketide moiety is released from a type II polyketides synthase must also be considered. The exact sequence in which the individual biosynthetic components of the variecolortides come together, and the mechanism of their fusion remain to be determined.

In summary, we have developed a concise total synthesis of variecolortide A and B that provides the natural racemates in seven and five steps, respectively (longest linear sequence). Our synthesis is largely devoid of protecting-group operations[15] and is highly convergent. It incorporates an unprecedented hetero-Diels–Alder reaction of a 1,4-anthraquinone with a didehydrodiketopiperazine to form the central spirocyclic core of the natural products. Density functional theory calculations strongly support our hypothesis that this key reaction proceeds through a concerted cycloaddition. Our synthesis also raises questions about whether a similar step could occur in nature and whether an enzyme is involved. Answering these questions will require detailed biosynthetic studies, which are beyond the scope of the present work. Meanwhile, our laboratory synthesis provides ample access to the variecolortides and allows for a full biological exploration of these fascinating natural products.

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Supporting Information

69451 Weinheim, Germany

Concise Total Syntheses of Variecolortides A and B through an Unusual Hetero-Diels–Alder Reaction**

Christian A. Kuttruff, Hendrik Zipse, and Dirk Trauner**

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Index:

General Experimental Details. Unless stated otherwise, all reactions were performed in oven-dried or flame-dried glassware under a positive pressure of nitrogen. Commercial reagents and solvents were used as received with the following exceptions. Tetrahydrofuran (THF) was distilled from benzophenone and sodium immediately prior to use. Triethylamine, diisopropylamine and diisopropylethylamine were distilled over calcium hydride immediately before use. Reactions were magnetically stirred and monitored by NMR spectroscopy or analytical thin-layer chromatography (TLC) using E. Merck 0.25 mm silica gel 60 F_{254} precoated glass plates. TLC plates were visualized by exposure to ultraviolet light (UV, 254 nm) and/or exposure to an aqueous solution of ceric ammoniummolybdate (CAM), an aqueous solution of potassium permanganate ($KMnO_4$), an acidic solution of vanillin or a solution of ninhydrin in ethanol followed by heating with a heat gun. Flash column chromatography was performed as described by Still *et al.* employing silica gel (60 Å, 40-63 μm, Merck) and a forced flow of eluant at 1.3–1.5 bar pressure.[1] Yields refer to chromatographically and spectroscopically (^{1}H and ^{13}C NMR) pure material.

Instrumentation. Proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded on Varian VNMRS 300, VNMRS 400, INOVA 400 or VNMRS 600 spectrometers. Proton chemical shifts are expressed in parts per million (δ scale) and are calibrated using residual undeuterated solvent as an internal reference ($CHCl_3$: δ 7.26, DMSO-d_6: δ 2.50, $(CD_3)_2CO$: δ 2.05). Data for ^{1}H NMR spectra are reported as follows: chemical shift (δ ppm) (multiplicity, coupling constant (Hz), integration). Multiplicities are reported as follows: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, *br* = broad, app = apparent, or combinations thereof. Carbon nuclear magnetic resonance (^{13}C NMR) spectra were recorded on Varian VNMRS 300, VNMRS 400, INOVA 400 or VNMRS 600 spectrometers. Carbon chemical shifts are expressed in parts per million (δ scale) and are referenced to the carbon resonances of the solvent ($CDCl_3$: δ 77.0, DMSO-d_6: δ 39.5, $(CD_3)_2CO$: δ 29.8). Infrared (IR) spectra were recorded on a Perkin Elmer Spectrum BX II (FTIR System). IR data is reported in frequency of absorption (cm^{-1}). Mass spectroscopy (MS) experiments were performed on a Thermo Finnigan MAT 95 (EI) or on a Thermo Finnigan LTQ FT (ESI) instrument.

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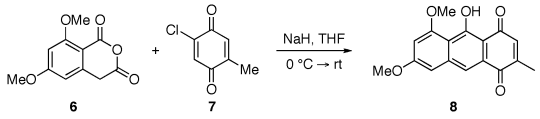
Synthetic procedures.



10-Hydroxy-5,7-dimethoxy-2-methyl-1,4-anthraquinone (8):

Anhydride **6**[2] (1.00 g, 4.50 mmol, 1.0 equiv) was added to a suspension of NaH (360 mg of a ca. 60 wt% suspension in mineral oil, 9.00 mmol, 2.0 equiv) in THF (40 mL) and cooled to 0 °C. After stirring the reaction mixture for 10 min at this temperature, a solution of 2-chloro-5-methyl-benzoquinone (**7**) (727 mg, 4.55 mmol, 1.01 equiv) in THF (30 mL) was added at 0 °C over 20 min, and the ensuing mixture was stirred for another 30 min at 0 °C. The resulting red suspension was allowed to warm to rt and a sat. aq. solution of NH_4Cl (70 mL) was added. The reaction mixture was extracted with CH_2Cl_2 (2 × 70 mL) and the combined organic layers were washed with water (100 mL), dried over sodium sulfate and concentrated *in vacuo*. Purification of the dark residue by flash column chromatography (silica gel, $CHCl_3$:acetone = 15:1) afforded quinone **8** (698 mg, 2.34 mmol, 52%) as a dark-purple solid.

TLC ($CHCl_3$:acetone = 10:1), R_f = 0.78 (UV/CAM)

M.p.: 242–245 °C

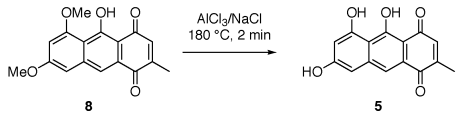
^{1}H NMR (600 MHz, $CDCl_3$) δ: 14.90 (*br* s, 1 H), 7.86 (s, 1 H), 6.85–6.84 (m, 2 H), 6.63 (d, J = 2.3 Hz, 1 H), 4.01 (s, 3 H), 3.95 (s, 3 H), 2.18 (d, J = 1.8 Hz, 3 H).

^{13}C NMR (150 MHz, $CDCl_3$) δ: 187.9, 184.8, 165.1, 162.5, 161.3, 149.2, 140.3, 137.4, 128.5, 121.4, 113.3, 108.0, 102.9, 101.3, 56.3, 55.7, 16.5.

IR (Diamond-ATR, neat) ν_{max}: 1656, 1633, 1594, 1571, 1336, 1214, 1165, 1115 cm^{-1}.

HRMS (ESI) calcd for $C_{17}H_{14}O_5$ $[M+Na]^+$: 321.0739; found: 321.0734.

[2] prepared according to Bauta, W. E.; Lovett, D. P.; Cantrell, W. R.; Burke, B. D. *J. Org. Chem.* **2003**, *68*, 5967–5973.



Hydroxyviocristin (5):

Quinone **8** (20.0 mg, 67.0 µmol, 1.00 equiv) was added rapidly to a melt of anhydrous $AlCl_3$ (2.00 g) and NaCl (0.5 g) at 140 °C. The dark blue reaction mixture was quickly heated to 180 °C (by transferring the reaction vessel into a preheated oil bath), stirred at this temperature for 2 min, cooled for 1 min and then poured into ice (200 mL) containing conc. hydrochlorid acid (10 mL). The purple mixture was heated to 50 °C for 1 h before being extracted with CH_2Cl_2 (3 × 30 mL). The combined organic layers were washed with water (2 × 10 mL), dried over sodium sulfate and concentrated *in vacuo.* Purification of the dark purple residue by reversed phase HPLC (50 to 95% MeOH in H_2O linear gradient over 41 min; preparative RP column, Dynamax Microsorb 60 C_{18}, 8 µm particle size) provided hydroxyviocristin (**5**) (13.6 mg, 50.3 µmol, 75%) as a dark purple solid, which was almost insoluble in most of the conventional solvents.

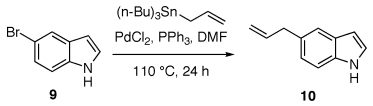
M.p.: 250 °C (decomp.)

^{1}H NMR (400 MHz, $(CD_3)_2CO$) δ: 16.17 (*br* s, 1 H), 10.41 (s, 1 H), 9.70 (s, 1 H), 7.78 (s, 1 H), 7.04 (s, 1 H), 7.00 (d, *J* = 2.0 Hz, 1 H), 6.61 (d, *J* = 1.9 Hz, 1 H), 2.17 (s, 3 H).

IR (Diamond-ATR, neat) ν_{max}: 3375, 1653, 1623, 1589, 1437, 1371, 1334, 1228, 1158, 887, 866, 833 cm^{-1}.

HRMS (EI) calcd for $C_{15}H_{10}O_5$ $[M]^{+\bullet}$: 270.0523; found: 270.0522.

Note: Due to the low solubility of the title compound in various deuterated solvents, a ^{13}C spectrum could not be recorded.



5-allyl-1*H*-indole (10):

To a solution of 5-bromoindole (**9**) (2.01 g, 10.3 mmol, 1.0 equiv) in degassed DMF (12 mL) in a pressure tube was added triphenylphosphine (544 mg, 2.05 mmol, 0.2 equiv), palladium(II)-chloride (91 mg, 0.51 mmol, 0.05 equiv) and allyltributylstannane (3.93 mL, 12.3 mmol, 1.2 equiv). The bright yellow reaction mixture was heated to 120 °C in a preheated oil bath. After 24

h, the black-green reaction mixture was cooled to rt and diethyl ether (25 mL) and sat. aq. NaCl (15 mL) were added sequentially. The organic layer was separated, washed with water (15 mL), dried over sodium sulfate and concentrated under reduced pressure. The resulting yellow oil was purified by flash column chromatography (silica gel, hexanes/EtOAc = 10:1) to afford indole **10** (1.46 g, 9.29 mmol, 90%) as a colourless oil.

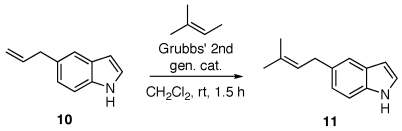
TLC (hexanes:EtOAc = 10:1), R_f = 0.39 (UV/vanillin)

^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.04 (*br* s, 1 H), 7.48–7.47 (m, 1 H), 7.33 (d, *J* = 8.3 Hz, 1 H), 7.20–7.17 (m, 1 H), 7.06 (dd, *J* = 1.6, 8.3 Hz, 1 H), 6.52–6.51 (m, 1 H), 6.07 (app. ddt, *J* = 6.7, 10.0, 16.8 Hz, 1 H), 5.12 (app. ddd, *J* = 1.7, 3.5, 17.0 Hz, 1 H), 5.09–5.05 (m, 1 H), 3.51 (d, *J* = 6.7 Hz, 2 H).

^{13}C NMR (100 MHz, $CDCl_3$) δ: 138.6, 134.5, 131.4, 128.1, 124.3, 123.1, 120.1, 115.0, 110.8, 102.3, 40.4.

IR (Diamond-ATR, neat) ν_{max}: 3408, 1637, 1474, 1414, 1333, 1090, 993, 911 cm^{-1}.

HRMS (EI) calcd for $C_{11}H_{11}N$ $[M]^{+\bullet}$: 157.0886; found: 157.0882.



10 **11**

5-(3-methylbut-2-enyl)-1*H*-indole (11):

To a solution of indole **10** (1.39 g, 8.81 mmol, 1.0 equiv) in degassed CH_2Cl_2 (10 mL) was added 2-methyl-2-butene (28.1 mL, 246 mmol, 30 equiv) at rt. Grubbs' 2nd generation catalyst (524 mg, 0.62 mmol, 0.07 equiv) was then added and the reaction mixture was stirred for 1.5 h at rt. The solution was concentrated *in vacuo* and the residue was purified by flash column chromatography (silica gel, gradient: hexanes:EtOAc = 15:1 → 10:1) to afford the title compound (1.29 g, 6.96 mmol, 79%) as a black oil.

TLC (hexanes:EtOAc = 10:1), R_f = 0.19 (UV/vanillin)

^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.06 (*br* s, 1 H), 7.45 (s, 1 H), 7.33–7.31 (m, 1 H), 7.18–7.16 (m, 1 H), 7.05–7.03 (m, 1 H), 6.50 (app. s, 1 H), 5.44–5.39 (m, 1 H), 3.45 (d, *J* = 7.3 Hz, 2 H), 1.77 (s, 6 H).

^{13}C NMR (100 MHz, $CDCl_3$) δ: 134.3, 133.2, 131.6, 128.1, 124.4, 124.2, 122.9, 119.6, 110.8, 102.3, 34.4, 25.8, 17.8.

IR (Diamond-ATR, neat) ν_{max}: 3409, 2913, 1474, 1451, 1415, 1330, 1090, 800 cm^{-1}.

HRMS (EI) calcd for $C_{13}H_{15}N$ $[M]^{+\bullet}$: 185.1199; found: 185.1203.

11 S1

3-chloro-5-(3-methylbut-2-enyl)-1*H*-indole S1:

To a solution of indole **11** (815 mg, 4.40 mmol, 1.0 equiv) in DMF (4 mL) was added *N*-chlorosuccinimide (606 mg, 4.4 mmol, 1.0 equiv) and the reaction mixture was stirred at rt. After 3.5 h, EtOAc (30 mL) and sat. aq. NaCl (20 mL) were added to the reaction mixture. The layers were separated, and the aqueous layer was extracted with EtOAc (3 × 20 mL). The combined organic layers were dried over sodium sulfate, filtered and concentrated under reduced pressure. The resulting oil was purified by flash column chromatography (silica gel, hexanes:EtOAc = 10:1) to provide indole **S1** (701 mg, 3.19 mmol, 73%) as a green-black oil.

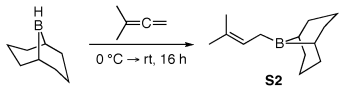
TLC (hexanes:EtOAc = 7:1), R_f = 0.34 (UV/vanillin)

^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.00 (*br* s, 1 H), 7.44–7.43 (m, 1 H), 7.28–7.25 (m, 1 H), 7.11–7.08 (m, 2 H), 5.45–5.38 (m, 1 H), 3.48 (d, *J* = 7.3 Hz, 2 H), 1.79–1.78 (m, 6 H).

^{13}C NMR (75 MHz, $CDCl_3$) δ: 134.1, 133.5, 132.0, 125.5, 124.1, 124.0, 120.9, 117.0, 111.3, 106.1, 34.4, 25.8, 17.8.

IR (Diamond-ATR, neat) ν_{max}: 3409, 2912, 1580, 1481, 1451, 1339, 1242, 1205, 1160, 1084, 1002 cm^{-1}.

HRMS (EI) calcd for $C_{13}H_{14}ClN$ $[M]^{+\bullet}$: 219.0809; found: 219.0816.

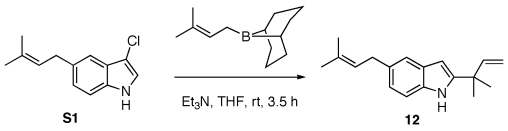


S2

Prenyl-9-BBN (S2):

A 50 mL flask was charged with 9-borabicyclo[3.3.1]nonane (0.5 M solution in THF, 20.6 mL, 10.3 mmol, 3.0 equiv) and cooled in an ice-bath. After the clear solution had turned cloudy, 3-methyl-1,2-butadiene (1.22 mL, 12.0 mmol, 3.5 equiv) was added and the flask was sealed tightly with a plastic yellow cap. The reaction mixture was warmed to rt and stirred at this

temperature for 16 h. This material was used without further purification in the next step of the reaction sequence.



5-(3-methylbut-2-enyl)-2-(2-methylbut-3-en-2-yl)-1*H*-indole (12):

To a solution of indole **S1** (756 mg, 3.44 mmol, 1.0 equiv) in THF (12 mL) was added triethylamine (1.55 mL, 11.2 mmol, 3.25 equiv) at rt. After 20 min, the freshly-prepared solution of prenyl-9-BBN (**S2**) (see above, ca. 10.3 mmol, 3.0 equiv) was slowly added at rt. After 3.5 h, the reaction mixture was concentrated *in vacuo* and the resulting residue was purified by flash column chromatography (silica gel, hexanes:EtOAc = 20:1) to afford the title compound (832 mg, 3.28 mmol, 96%) as a yellow oil.

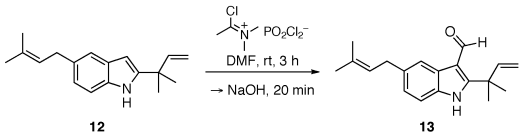
TLC (hexanes:EtOAc = 10:1), R_f = 0.48 (UV/vanillin)

^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.79 (*br* s, 1 H), 7.35 (app. dd, *J* = 0.7, 1.5 Hz, 1 H), 7.21 (d, *J* = 8.2 Hz, 1 H), 6.97 (dd, *J* = 1.7, 8.2 Hz, 1 H), 6.26 (app. dd, *J* = 0.7, 2.2 Hz, 1 H), 6.08–6.01 (m, 1 H), 5.42–5.37 (m, 1 H), 5.14–5.12 (m, 1 H), 5.10–5.09 (m, 1 H), 3.42 (d, *J* = 7.4 Hz, 2 H), 1.76–1.75 (m, 6 H), 1.48 (s, 6 H).

^{13}C NMR (100 MHz, $CDCl_3$) δ: 146.1, 146.1, 145.9, 134.4, 133.0, 131.4, 128.8, 124.6, 122.1, 119.1, 112.1, 110.2, 97.7, 38.2, 34.4, 27.4, 25.8, 17.8.

IR (Diamond-ATR, neat) ν_{max}: 3424, 2968, 2913, 1479, 1453, 1293, 997, 915 cm^{-1}.

HRMS (EI) calcd for $C_{18}H_{23}N$ $[M]^{+\bullet}$: 253.1825; found: 253.1823.



Formyl indole 13:

A Schlenk tube was charged with DMF (0.15 mL) and cooled to 0 °C. $POCl_3$ (0.05 mL, 0.58 mmol, 1.3 equiv) was added dropwise and the reaction mixture was stirred at 0 °C. After 20 min, a solution of indole **12** (112 mg, 0.44 mmol, 1.0 equiv) in DMF (2 mL) was slowly added to the

pre-formed Vilsmeier reagent and the ensuing solution was stirred at rt. After 3 h the reaction was quenched with water (1 mL), and 2 M NaOH was added until pH = 8 was reached. The reaction mixture was stirred at 60 °C for 20 min, diluted with water and subsequently extracted with EtOAc (3 × 20 mL). The combined organic layers were washed with 10% LiCl solution (10 mL), dried over sodium sulfate and concentrated under reduced pressure. The residue was purified by flash column chromatography (silica gel, hexanes:EtOAc = 3:1) to afford formyl indole **13** (105 mg, 0.37 mmol, 84%) as an off-white solid.

TLC (hexanes:EtOAc = 5:1), R_f = 0.16 (UV/CAM)

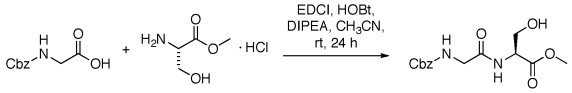
M.p.: 121–125 °C

^{1}H NMR (600 MHz, $CDCl_3$) δ: 10.44 (s, 1 H), 8.73 (*br* s, 1 H), 8.19 (s, 1 H), 7.28 (d, *J* = 8.4 Hz, 1 H), 7.08 (dd, *J* = 1.7, 8.3 Hz, 1 H), 6.22 (dd, *J* = 10.5, 17.5 Hz, 1 H), 5.38–5.35 (m, 1 H), 5.28–5.27 (m, 1 H), 5.25 (s, 1 H), 3.44 (d, *J* = 7.3 Hz, 2 H), 1.75–1.73 (m, 6 H), 1.67 (s, 6 H).

^{13}C NMR (150 MHz, $CDCl_3$) δ: 186.5, 154.9, 145.0, 137.0, 132.3, 131.8, 127.4, 124.2, 124.1, 121.4, 113.9, 113.7, 110.9, 110.7, 39.7, 34.6, 28.8, 25.8, 17.9.

IR (Diamond-ATR, neat) ν_{max}: 3160, 2970, 1616, 1584, 1442, 1370 cm^{-1}.

HRMS (EI) calcd for $C_{19}H_{23}NO$ $[M]^{+\bullet}$: 281.1774; found: 281.1773.



14 **15** **16**

Methyl Z-glycyl-L-serinate (16):

To a solution of Z-glycine (**14**) (5.00 g, 23.9 mmol, 1.0 equiv) in CH_3CN (100 mL) was added L-serine methyl ester hydrochloride (**15**) (5.00 g, 23.9 mmol, 1.0 equiv). The suspension was cooled to 0 °C and EDC hydrochloride (5.49 g, 28.7 mmol, 1.2 equiv), HOBt (3.88 g, 28.7 mmol, 1.2 equiv) and DIPEA (16.5 mL, 95.6 mmol, 4.0 equiv) were added. The reaction mixture was warmed to rt and stirred at this temperature for 24 h. The solvent was removed under reduced pressure and the resulting colourless oil was dissolved in CH_2Cl_2 (200 mL), washed with 1 M HCl (1 × 50 mL), sat. aq. $NaHCO_3$ (1 × 50 mL), sat. aq. NaCl (1 × 50 mL), dried over sodium sulfate, and concentrated *in vacuo*. The residue so obtained was purified by flash column chromatography (silica gel, CH_2Cl_2:MeOH = 20:1) furnishing amide **16** (5.72 g, 18.4 mmol, 77%) as a white foam.

TLC (CH_2Cl_2:MeOH = 10:1), R_f = 0.42 (UV/ninhydrin)

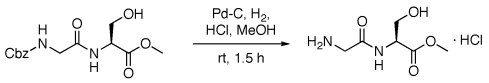
M.p.: 94-95 °C

^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.15 (d, J = 7.8 Hz, 1 H), 7.45 (t, J = 6.1 Hz, 1 H), 7.39 – 7.27 (m, 5 H), 5.09 (t, J = 5.7 Hz, 1 H), 5.03 (s, 2 H), 4.39 – 4.35 (m, 1 H), 3.75 – 3.65 (m, 3 H), 3.65 – 3.57 (m, 4 H).

^{13}C NMR (100 MHz, DMSO-d_6) δ: 171.0, 169.3, 156.5, 137.0, 128.3, 127.8, 127.7, 65.4, 61.3, 54.5, 51.9, 43.2.

IR (Diamond-ATR, neat) ν_{max}: 3381, 3299, 2951, 1736, 1719, 1690, 1544, 1518, 1225 cm^{-1}.

HRMS (ESI) calcd for $C_{14}H_{18}N_2O_6$ $[M+Na]^+$: 333.1057; found: 333.1056.



16 **17**

Glycyl-L-serine Methyl Ester Hydrochloride (17):

To a solution of amide **16** (3.10 g, 9.99 mmol, 1.0 equiv) in degassed MeOH (60 mL) was added 10% Pd/C (1.00 g, 0.94 mmol, 0.09 equiv) and conc. HCl (0.85 mL). The inner atmosphere of the flask was exchanged three times with hydrogen and the resulting mixture was stirred under hydrogen atmosphere (double layer balloon) for 1.5 h at rt. The reaction mixture was filtered through a pad of Celite and the solvent was removed under reduced pressure. The residue was kept under high-vacuum overnight to provide a white foam which was triturated with absolute diethyl ether to yield dipeptide **17** (1.85 g, 8.71 mmol, 87%) as a very hygroscopic white solid.

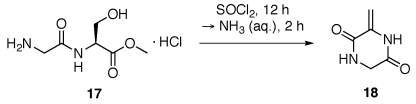
M.p.: 48-50 °C

^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.98 (d, J = 7.5 Hz, 1 H), 8.24 (*br* s, 3 H), 5.27 (*br* s, 1 H), 4.39 (app. dt, J = 4.6, 7.6 Hz, 1 H), 3.75–3.62 (m, 2 H), 3.64 (s, 3 H), 3.60 (d, 1.5 Hz, 2 H).

^{13}C NMR (100 MHz, DMSO-d_6) δ: 170.5, 166.1, 61.1, 54.9, 52.0, 40.2.

IR (Diamond-ATR, neat) ν_{max}: 3280, 2953, 1730, 1665, 1567, 1345, 1225, 1063 cm^{-1}.

HRMS (ESI) calcd for $C_6H_{12}N_2O_4$ $[M+H]^+$: 177.0870, found 177.0868.



17 18

3-methylene-piperazine-2,5-dione (18):

To amide **17** (1.67 g, 7.88 mmol, 1.0 equiv) was added thionyl chloride (6.0 mL, 82.7 mmol, 10.5 equiv) at 0 °C. The reaction mixture was warmed to rt and stirred for 12 h. To the clear yellow solution was added absolute diethyl ether (30 mL) and the ensuing suspension was stirred for 15 min at rt. The very hygroscopic white precipitate was filtered off under Argon atmosphere and dried under high vacuum for 7 h. The white solid was dissolved in absolute MeOH (3 mL) and cooled to 0 °C. To the clear yellow solution was slowly added diethyl ether (25 mL) and the suspension was kept at this temperature for 2 h. The formed white precipitate was filtered, dried under high vacuum and subsequently dissolved in aq. ammonia (12 mL), which led to the formation of a white precipitate. After 2 h, the reaction mixture was concentrated under reduced pressure and the white residue was purified by recrystallization from water to afford the title compound (570 mg, 4.52 mmol, 57%) as a white solid.

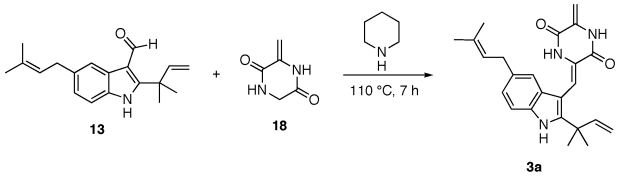
M.p.: 247 °C (decomp.)

^{1}H NMR (400 MHz, DMSO-d_6) δ: 10.51 (*br* s, 1 H), 8.21 (*br* s, 1 H), 5.17 (s, 1 H), 4.77 (d, J = 0.6 Hz, 1 H), 3.95 (d, J = 2.0 Hz, 2 H).

^{13}C NMR (100 MHz, DMSO-d_6) δ: 163.6, 158.0, 134.8, 99.0, 44.9.

IR (Diamond-ATR, neat) ν_{max}: 3189, 3077, 1681, 1630, 1455, 1399, 1326 cm^{-1}.

HRMS (EI) calcd for $C_5H_6N_2O_2$ $[M]^{+\bullet}$: 126.0424, found 126.0422.



13 18 3a

Isoechinulin B (3a):

A pressure tube was charged with aldehyde **13** (40.0 mg, 142 µmol, 1.0 equiv) and diketopiperazine **18** (44.8 mg, 355 µmol, 2.5 equiv) and freshly distilled piperidine (1.5 mL). The pressure tube was sealed under argon atmosphere and heated to 110 °C. After 7 h, the reaction mixture was cooled to rt and concentrated *in vacuo*. The residue was purified by flash

column chromatography (silica gel, $CHCl_3$:acetone = 150:1 → 10:1 → 3:1) to yield isoechinulin B (**3a**) (21.5 mg, 55.2 µmol, 39%) as a yellow waxy solid as well as recovered aldehyde **13** (7 mg, 24.9 µmol, 18%).

TLC ($CHCl_3$:acetone = 5:1), R_f = 0.61 (UV/CAM)

^{1}H NMR (600 MHz, $CDCl_3$) δ: 8.70 (s, 1 H), 8.28 (s, 1 H), 7.66 (s, 1 H), 7.28–7.27 (m, 1 H), 7.26–7.25 (m, 1 H), 7.07 (s, 1 H), 7.04 (dd, *J* = 1.6, 8.3 Hz, 1 H), 6.06 (dd, *J* = 10.6, 17.4 Hz, 1 H), 5.60 (d, *J* = 1.2 Hz, 1 H), 5.36–5.33 (m, 1 H), 5.22 (dd, *J* = 0.9, 10.5 Hz, 1 H), 5.18 (dd, *J* = 0.9, 17.4 Hz, 1 H), 4.98–4.97 (m, 1 H), 3.42 (d, *J* = 7.3 Hz, 2 H), 1.72–1.71 (m, 6 H), 1.52 (s, 6 H).

^{13}C NMR (150 MHz, $CDCl_3$) δ: 157.7, 155.7, 144.4, 144.2, 135.0, 133.6, 132.8, 132.2, 126.1, 124.2, 124.0, 123.4, 118.0, 113.4, 113.3, 111.2, 102.8, 101.9, 39.3, 34.5, 27.4, 25.7, 17.8.

IR (Diamond-ATR, neat) ν_{max}: 3354, 3021, 2969, 2918, 1966, 1721, 1677, 1641, 1376, 1247, 1149, 803 cm^{-1}.

HRMS (ESI) calcd for $C_{24}H_{28}N_3O_2$ $[M+H]^+$: 390.2176; found: 390.2176.

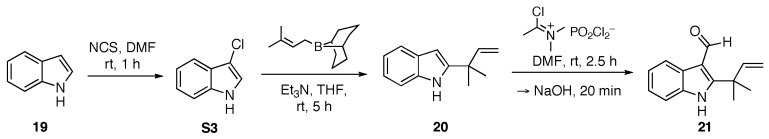
Table S1. ^{1}H NMR data comparison between reported natural isoechinulin B (**3a**) and synthetic isoechinulin B (**3a**).

Literature report[3] (^{1}H, MHz*, $(CD_3)_2CO$)	This report (^{1}H, 400 MHz, $(CD_3)_2CO$)
10.23 (*br* s, 1 H)	10.28 (*br* s, 1 H)
9.80 (*br* s, 1 H)	9.78 (*br* s, 1 H)
8.08 (*br* s, 1 H)	8.11 (*br* s, 1 H)
7.30 (d, *J* = 8.2 Hz, 1 H)	7.31 (d, *J* = 8.2 Hz, 1 H)
7.2–7.0 (*br* s, 2 H)	7.15–7.14 (m, 1 H), 7.13 (s, 1 H)
6.96 (*br* d, *J* = 8.2 Hz, 1 H)	6.98 (dd, *J* = 1.6, 8.3 Hz, 1 H)
6.13 (dd, *J* = 10.5, 18.0 Hz, 1 H)	6.14 (dd, *J* = 10.6, 17.4 Hz, 1 H)
5.4–5.0 (m, 5 H)	5.38 (s, 1 H), 5.37–5.33 (m, 1 H), 5.12 (dd, *J* = 1.1, 10.4 Hz, 1 H), 5.08 (dd, *J* = 1.1, 3.4 Hz, 1 H), 5.03 (d, *J* = 0.8 Hz, 1 H)
3.38 (d, *J* = 8.0 Hz, 2 H)	3.39 (d, *J* = 7.5 Hz, 2 H)

[3] Nagasawa, H.; Isogai, A.; Suzuki, A.; Tamura, S. *Tetrahedron Lett.* **1976**, *17*, 1601–1604.

1.68 (s, 6 H)	1.70–1.69 (m, 6 H)
1.54 (s, 6 H)	1.56 (s, 6 H)

Note: * = the corresponding data is not provided in the original isolation paper.



Formyl indole 21:

Note: the following reactions were carried out under the exclusion of light.

To a solution of indole (**19**) (1.00 g, 8.54 mmol, 1.0 equiv) in DMF (35 mL) was added *N*-chlorosuccinimide (1.23 g, 8.96 mmol, 1.05 equiv) at rt. After 1 h, sat. aq. NaCl (40 mL) was added and the aqueous layer was extracted with EtOAc (3 × 35 mL). The combined organic layers were washed with water (50 mL), dried over sodium sulfate and concentrated *in vacuo*. Purification of the yellow-brown residue by flash column chromatography (silica gel, hexanes:EtOAc = 5:1) afforded indole **S3** (1.17 g, 7.73 mmol, 91%) as a yellow-green solid.

Et_3N (3.49 mL, 25.1 mmol, 3.25 equiv) was added dropwise to a solution of **S3** (1.17 g, 7.73 mmol, 1.0 equiv) in THF (25 mL) at rt. After 25 min, a freshly prepared solution of prenyl-9-BBN (**S2**) (5.14 g, 27.1 mmol, 3.5 equiv) in THF (46 mL) was added to give a clear, bright-yellow reaction mixture, which was stirred for 5 h at rt. The solvent was removed under reduced pressure and the brown residue was purified by flash column chromatography (silica gel, hexanes:EtOAc = 30:1) to give indole **20** (1.06 g, 5.70 mmol, 74%) as a yellow oil.

A Schlenk tube was charged with DMF (2.30 mL, 29.9 mmol, 5.2 equiv) and cooled to 0 °C. $POCl_3$ (0.69 mL, 7.42 mmol, 1.3 equiv) was added dropwise and the reaction mixture was stirred at 0 °C. After 30 min, a solution of indole **20** (1.06 g, 5.71 mmol, 1.0 equiv) in DMF (25 mL) was added dropwise to the Vilsmeier reagent and the solution was stirred at rt. After 2.5 h the reaction mixture was cooled to 0 °C and the pH was adjusted to pH = 9 by addition of 2 M NaOH. The solution was extracted with EtOAc (3 × 100 mL) and the combined organic layers were washed with 10% LiCl solution (4 × 70 mL) and dried over sodium sulfate. The solvent was removed *in vacuo* and the residue was dried under high-vacuum to give formyl indole **21**

(1.14 g, 5.33 mmol, 93%) as an off-white solid which was analytically pure without chromatography.

TLC (hexanes:EtOAc = 1:1), R_f = 0.61 (UV/CAM)

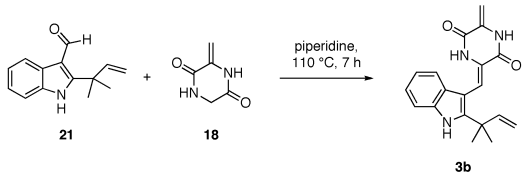
M.p.: 186 °C

^{1}H NMR (400 MHz, $CDCl_3$) δ: 10.47 (s, 1 H), 8.94 (*br* s, 1 H), 8.38–8.36 (m, 1 H), 7.40–7.38 (m, 1 H), 7.30–7.22 (m, 2 H), 6.24 (dd, *J* = 10.6, 17.4 Hz, 1 H), 5.30 (d, *J* = 6.9 Hz, 1 H), 5.26 (s, 1 H), 1.69 (s, 6 H).

^{13}C NMR (100 MHz, $CDCl_3$) δ: 186.6, 155.0, 144.9, 133.8, 127.1, 123.5, 123.0, 122.0, 113.9, 113.9, 110.9, 39.7, 28.9.

IR (Diamond-ATR, neat) ν_{max}: 3162, 3120, 3091, 2970, 2930, 2853, 2812, 1620, 1582, 1441, 1372, 1305, 1246, 1178, 1154, 932, 791, 660 cm^{-1}.

HRMS (ESI) calcd for $C_{14}H_{16}NO$ $[M+H]^+$: 214.1226; found: 214.1226.



21 18 3b

Neoechinulin B (3b):

A pressure tube was charged with aldehyde **21** (40.0 mg, 188 µmol, 1.0 equiv), diketopiperazine **18** (59.1 mg, 469 µmol, 2.5 equiv) and freshly distilled piperidine (1.5 mL). The pressure tube was sealed under argon atmosphere and heated to 110 °C. After 7 h, the reaction mixture was cooled to rt and concentrated *in vacuo*. The residue was purified by flash column chromatography (silica gel, gradient: $CHCl_3$:acetone = 40:1 → 10:1 → 5:1) to yield neoechinulin B (**3b**) (22.0 mg, 68.5 µmol, 36%) as a yellow solid as well as recovered aldehyde **21** (3.1 mg, 24.9 µmol, 8%).

TLC (hexanes:EtOAc = 1:1), R_f = 0.27 (UV/CAM)

M.p.: 247–250 °C

^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.52 (*br* s, 1 H), 8.35 (*br* s, 1 H), 7.65 (*br* s, 1 H), 7.37 (d, *J* = 7.1 Hz, 1 H), 7.32 (d, *J* = 7.0 Hz, 1 H), 7.28 (*br* s, 1 H), 7.24–7.16 (m, 2 H), 6.08 (dd, *J* = 10.6, 17.4 Hz, 1 H), 5.62 (d, *J* = 1.7 Hz, 1 H), 5.24 (dd, *J* = 0.8, 10.6 Hz, 1 H), 5.20 (dd, *J* = 0.7, 17.4 Hz, 1 H), 4.97 (s, 1 H), 1.54 (s, 6 H).

^{13}C NMR (100 MHz, $CDCl_3$) δ: 157.5, 155.8, 144.3, 144.1, 134.3, 133.5, 125.9, 124.5, 122.5, 121.3, 119.0, 113.5, 113.1, 111.3, 103.0, 102.1, 39.3, 27.4.

IR (Diamond-ATR, neat) ν_{max}: 3356, 2969, 2916, 1674, 1642, 1614, 1522, 1420, 1389, 1309, 1244, 995, 916, 877 cm^{-1}.

HRMS (ESI) calcd for $C_{19}H_{20}N_3O_2$ $[M+H]^+$: 322.1550; found: 322.1550.

Table S2. ^{1}H NMR Data comparison between reported natural neoechinulin B (**3b**) and synthetic neoechinulin B (**3b**).

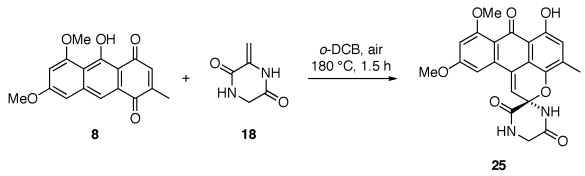
Literature report[4] (^{1}H, 400 MHz, $CDCl_3$)	This report (^{1}H, 400 MHz, $CDCl_3$)
-	8.52 (*br* s, 1 H)
-	8.35 (*br* s, 1 H)
-	7.65 (*br* s, 1 H)
7.37 (d, *J* = 6.8 Hz, 1 H)	7.37 (d, *J* = 7.1 Hz, 1 H)
7.31 (d, *J* = 6.8 Hz, 1 H)	7.32 (d, *J* = 7.0 Hz, 1 H)
7.27 (s, 1 H)	7.28 (*br* s, 1 H)
7.23–7.15 (m, 2 H)	7.24–7.16 (m, 2 H)
6.07 (dd, *J* = 11.9, 17.4 Hz, 1 H)	6.08 (dd, *J* = 10.6, 17.4 Hz, 1 H)
5.59 (d, *J* = 1.6 Hz, 1 H)	5.62 (d, *J* = 1.7 Hz, 1 H)
5.23 (dd, *J* = 0.8, 11.9 Hz, 1 H)	5.24 (dd, *J* = 0.8, 10.6 Hz, 1 H)
5.20 (dd, *J* = 0.8, 17.4 Hz, 1 H)	5.20 (dd, *J* = 0.7, 17.4 Hz, 1 H)
4.93 (d, *J* = 1.6 Hz, 1 H)	4.97 (s, 1 H)
1.53 (s, 6 H)	1.54 (s, 6 H)

Table S3. ^{13}C NMR Data comparison between reported natural neoechinulin B (**3b**) and synthetic neoechinulin B (**3b**).

Literature report (^{13}C, 100 MHz, $CDCl_3$)	This report (^{13}C, 100 MHz, $CDCl_3$)
157.4	157.5
155.8	155.8
144.3	144.3
144.1	144.1

[4] Kuramochi, K.; Aoki, T.; Nakazaki, A.; Kamisuki, S.; Takeno, M.; Ohnishi, K.; Kimoto, K.; Watanabe, N.; Kamakura, T.; Arai, T.; Sugawara, F.; Kobayashi, S. *Synthesis* **2008**, *23*, 3810–3818.

134.3	134.3
133.5	133.5
125.8	125.9
124.4	124.5
122.5	122.5
121.3	121.3
119.0	119.0
113.5	113.5
113.2	113.1
111.3	111.3
103.0	103.0
102.0	102.1
39.2	39.3
27.3	27.4



9,10-anthraquinone methide (25):

Note: no precautions were taken to exclude oxygen from the reaction vessel.

To a mixture of quinone **8** (50.1 mg, 168 µmol, 1.0 equiv) and diketopiperazine **18** (23.3 mg, 185 µmol, 1.1 equiv) in a pressure tube was added *o*-dichlorobenzene (1.5 mL) and the dark purple reaction mixture was heated to 180 °C. After 1.5 h, the mixture was cooled to rt and subjected directly to flash column chromatography (silica gel, eluting first with hexanes to remove *o*-dichlorobenzene, then gradient: $CHCl_3$:acetone = 10:1 → $CHCl_3$:MeOH 5:1) to afford the title compound (52 mg, 123 µmol, 73%) as a brown solid.

TLC ($CHCl_3$:MeOH = 10:1), R_f = 0.33 (UV/CAM)

M.p.: 220 °C (decomp.)

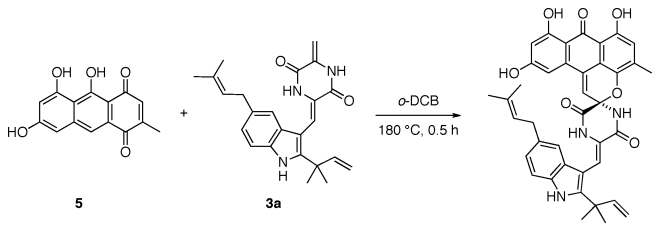
^{1}H NMR (400 MHz, DMSO-d_6) δ: 13.02 (s, 1 H), 9.48 (s, 1 H), 8.69 (d, *J* = 3.9 Hz, 1 H), 7.34 (d, *J* = 2.3 Hz, 1 H), 7.17 (s, 1 H), 6.86 (d, *J* = 0.8 Hz, 1 H), 6.77 (d, *J* = 2.2 Hz, 1 H), 4.24 (d, *J*

= 18.0 Hz, 1 H), 3.98 (s, 3 H), 3.91 (s, 3 H), 3.82 (app. dd, *J* = 4.1, 18.0 Hz, 1 H), 2.23 (d, *J* = 0.8 Hz, 3 H).

^{13}C NMR (100 MHz, DMSO-d_6) δ: 186.4, 167.3, 164.5, 163.8, 163.3, 156.2, 138.0, 137.7, 134.3, 126.2, 120.6, 119.0, 116.7, 112.4, 110.4, 110.5, 100.2, 83.5, 56.2, 56.0, 44.6, 15.8.

IR (Diamond-ATR, neat) ν_{max}: 3200, 3102, 2924, 2851, 1688, 1598, 1488, 1410, 1330, 1247, 1212, 1164, 1021, 832 cm^{-1}.

HRMS (ESI) calcd for $C_{22}H_{19}N_2O_7$ $[M+H]^+$: 423.1187; found: 423.1186.



variecolortide A (**1a**)

Variecolortide A (1a):

Note: no precautions were taken to exclude oxygen from the reaction vessel.

To a mixture of hydroxyviocristin (**5**) (9.40 mg, 24.1 μmol, 1.0 equiv) and isoechinulin B (**3a**) (7.17 mg, 26.5 μmol, 1.0 equiv) in a pressure tube was added *o*-dichlorobenzene (1.5 mL) and the dark purple reaction mixture was heated to 180 °C (in a preheated oil bath). After 30 min, the reaction mixture was cooled to rt and subjected directly to flash column chromatography (silica gel, eluting first with hexanes to remove *o*-dichlorobenzene, then gradient: $CHCl_3$:acetone = 40:1 → 20:1 → 10:1→ 1:1) to afford variecolortide A (**1a**) (7.6 mg, 11.6 μmol, 48%) as a yellow solid.

TLC ($CHCl_3$:acetone = 2:1), R_f = 0.54 (UV/CAM)

M.p.: 178 °C (decomp.)

^{1}H NMR (600 MHz, DMSO-d_6) δ: 12.71 (s, 1 H), 11.81 (s, 1 H), 11.07 (s, 1 H), 11.04 (s, 1 H), 9.96 (s, 1 H), 9.52 (s, 1 H), 7.34 (d, *J* = 8.2 Hz, 1 H), 7.31 (*br* s, 1 H), 7.19–7.18 (m, 2 H), 7.01 (s, 1 H), 6.96 (d, *J* = 0.7 Hz, 1 H), 6.93 (dd, *J* = 1.6, 8.2 Hz, 1 H), 6.45 (d, *J* = 2.1 Hz, 1 H), 6.09 (dd, *J* = 10.3, 17.7 Hz, 1 H), 5.24 (*br* t, *J* = 7.2 Hz, 1 H), 5.08 (d, *J* = 17.6 Hz, 1 H), 5.08 (d, *J* = 10.3 Hz, 1 H), 3.32 (d, *J* = 7.2 Hz, 1 H), 3.29 (d, *J* = 7.2 Hz, 1 H), 2.24 (s, 3 H), 1.52 (s, 3 H), 1.51 (s, 3 H), 1.47 (s, 3 H), 1.45 (s, 3 H).

^{13}C NMR (150 MHz, DMSO-d_6) δ: 189.5, 165.3, 164.9, 161.8, 161.3, 156.1, 145.2, 145.1, 138.9, 136.4, 136.4, 133.6, 132.5, 130.6, 126.6, 126.1, 124.3, 123.2, 121.7, 119.8, 119.4, 118.8, 117.5, 114.8, 111.9, 111.3, 109.0, 107.4, 103.9, 103.7, 103.4, 83.9, 39.5, 34.2, 28.0, 27.4, 25.2, 17.4, 15.8.

IR (Diamond-ATR, neat) ν_{max}: 3189, 2921, 1687, 1604, 1470, 1359, 1285, 1233, 1215, 1023, 995 cm^{-1}.

HRMS (ESI) calcd for $C_{39}H_{34}N_3O_7$ $[M-H]^-$: 656.2404; found: 656.2390.

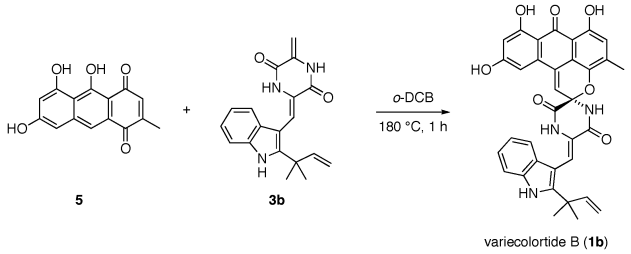
Table S4. ^{1}H NMR data comparison between reported natural variecolortide A (**1a**) and synthetic variecolortide A (**1a**).

Literature report[5] (^{1}H, 600 MHz, DMSO-d_6)	This report (^{1}H, 600 MHz, DMSO-d_6)
12.72 (s, 1 H)	12.71 (s, 1 H)
11.85 (s, 1 H)	11.81 (s, 1 H)
11.08 (s, 1 H)	11.07 (s, 1 H)
-	11.04 (s, 1 H)
10.01 (s, 1 H)	9.96 (s, 1 H)
9.54 (s, 1 H)	9.52 (s, 1 H)
7.34 (d, *J* = 8.4 Hz, 1 H)	7.34 (d, *J* = 8.2 Hz, 1 H)
7.31 (*br* s, 1 H)	7.31 (*br* s, 1 H)
7.18 (s, 1 H)	7.19–7.18 (m, 2 H)
7.17 (d, *J* = 1.9 Hz, 1 H)	
7.02 (s, 1 H)	7.01 (s, 1 H)
6.96 (s, 1 H)	6.96 (d, *J* = 0.7 Hz, 1 H)
6.93 (dd, *J* = 1.8, 8.4 Hz, 1 H)	6.93 (dd, *J* = 1.6, 8.2 Hz, 1 H)
6.44 (d, *J* = 1.9 Hz, 1 H)	6.45 (d, *J* = 2.1 Hz, 1 H)
6.09 (dd, *J* = 10.4, 17.4 Hz, 1 H)	6.09 (dd, *J* = 10.3, 17.7 Hz, 1 H)
5.24 (*br* t, *J* = 7.3 Hz, 1 H)	5.24 (*br* t, *J* = 7.2 Hz, 1 H)
5.09 (d, *J* = 10.4 Hz, 1 H)	5.09 (d, *J* = 10.3 Hz, 1 H)
5.08 (d, *J* = 17.4 Hz, 1 H)	5.08 (d, *J* = 17.6 Hz, 1 H)
3.32 (dd, *J* = 7.3, 15.0 Hz, 1 H)	3.30 (d, *J* = 7.2 Hz, 15.7 Hz, 1 H)
3.28 (dd, *J* = 7.3, 15.0 Hz, 1 H)	3.27 (d, *J* = 7.2 Hz, 15.7 Hz, 1 H)
2.24 (s, 3 H)	2.24 (s, 3 H)
1.52 (s, 3 H)	1.52 (s, 3 H)
1.50 (s, 3 H)	1.51 (s, 3 H)
1.47 (s, 3 H)	1.47 (s, 3 H)
1.44 (s, 3 H)	1.45 (s, 3 H)

Table S5. ^{13}C NMR data comparison between reported natural variecolortide A (**1a**) and synthetic variecolortide A (**1a**).

Literature report[5] (^{13}C, 150 MHz, DMSO-d_6)	This report (^{13}C, 150 MHz, DMSO-d_6)
189.8	189.5
165.4	165.3
165.0	164.9
162.0	161.8
161.4	161.3
156.2	156.1
145.3	145.2
145.1	145.1
139.0	138.9
136.4	136.4
136.4	136.4
133.7	133.6
132.5	132.5
130.7	130.6
126.6	126.6
126.2	126.1
124.4	124.3
123.2	123.2
121.8	121.7
119.8	119.8
119.4	119.4
118.9	118.8
117.5	117.5
114.9	114.8
111.9	111.9
111.4	111.3
109.0	109.0
107.4	107.4
103.9	103.9
103.8	103.7
103.5	103.4
84.0	83.9
39.0	39.5
34.3	34.2

28.0	28.0
27.4	27.4
25.3	25.2
17.5	17.4
15.8	15.8



Variecolortide B (1b):

Note: no precautions were taken to exclude oxygen from the reaction vessel.

To a mixture of hydroxyviocristin (**5**) (14.2 mg, 52.7 µmol, 1.1 equiv) and neoechinulin B (**3b**) (15.4 mg, 47.9 µmol, 1.0 equiv) in a pressure tube was added *o*-dichlorobenzene (1.5 mL) and the dark purple reaction mixture was heated to 180 °C (in a preheated oil bath). After 1 h, the reaction mixture was cooled to rt and subjected directly to flash column chromatography (silica gel, eluting first with hexanes to remove *o*-dichlorobenzene, then gradient: $CHCl_3$:acetone = 40:1 → 20:1 → 10:1→ 1:1) to afford variecolortide B (**1b**) (9.0 mg, 15.3 µmol, 32%) as an orange solid.

TLC ($CHCl_3$:acetone = 4:1), R_f = 0.29 (UV/CAM)

^{1}H NMR (600 MHz, $CDCl_3$) δ: 12.71 (s, 1 H), 11.82 (s, 1 H), 11.15 (s, 1 H), 11.07 (s, 1 H), 10.01 (s, 1 H), 9.57 (s, 1 H), 7.46–7.44 (m, 2 H), 7.18 (s, 1 H), 7.17 (d, *J* = 2.2 Hz, 1 H), 7.10 (app. ddd, *J* = 1.2, 7.0, 8.2 Hz, 1 H), 7.05 (app. ddd, *J* = 1.1, 7.1, 7.9 Hz, 1 H), 7.00 (s, 1 H), 6.97 (d, *J* = 0.8 Hz, 1 H), 6.46 (d, *J* = 2.2 Hz, 1 H), 6.11 (dd, *J* = 10.3, 17.6 Hz, 1 H), 5.09 (dd, *J* = 1.2, 17.6 Hz, 1 H), 5.09 (dd, *J* = 1.2, 10.3 Hz, 1 H), 2.28 (d, *J* = 0.7 Hz, 3 H), 1.53 (s, 3 H), 1.49 (s, 3 H).

^{13}C NMR (150 MHz, $CDCl_3$) δ: 189.5, 165.2, 164.9, 161.4, 161.3, 156.1, 145.1, 145.0, 138.8, 136.3, 136.2, 135.2, 126.3, 126.0, 123.6, 120.8, 119.8, 119.4, 119.3, 117.3, 114.7, 111.8, 111.6, 109.0, 107.4, 103.9, 103.6, 103.4, 84.0, 27.9, 27.4, 15.9

IR (Diamond-ATR, neat) ν_{max}: 3248, 2924, 1683, 1604, 1469, 1363, 1286, 1233, 1215, 1120, 1023, 819, 745 cm^{-1}.

HRMS (ESI) calcd for $C_{34}H_{26}N_3O_7$ $[M-H]^-$: 588.1776; found: 588.1763.

Table S6. ^1H NMR data comparison between reported natural variecolortide B (**1b**) and synthetic variecolortide B (**1b**).

Literature report[5] (^1H, 600 MHz, DMSO-d_6)	This report (^1H, 600 MHz, DMSO-d_6)
12.72 (s, 1 H)	12.71 (s, 1 H)
11.85 (s, 1 H)	11.82 (s, 1 H)
11.18 (s, 1 H)	11.15 (s, 1 H)
-	11.07 (s, 1 H)
10.06 (s, 1 H)	10.01 (s, 1 H)
9.59 (s, 1 H)	9.57 (s, 1 H)
7.45 (d, *J* = 7.8 Hz, 1 H); 7.45 (d, *J* = 8.0 Hz, 1 H)	7.46–7.44 (m, 2 H)
7.18 (s, 1 H)	7.18 (s, 1 H)
7.16 (d, *J* = 1.5 Hz, 1 H)	7.17 (d, *J* = 2.2 Hz, 1 H)
7.10 (dd, *J* = 7.0, 8.0 Hz, 1 H)	7.10 (ddd, *J* = 1.2, 7.0, 8.2 Hz, 1 H)
7.05 (dd, *J* = 7.0, 7.8 Hz, 1 H)	7.05 (ddd, *J* = 1.1, 7.1, 7.9 Hz, 1 H)
7.00 (s, 1 H)	7.00 (s, 1 H)
6.96 (s, 1 H)	6.97 (d, *J* = 0.8 Hz, 1 H)
6.43 (d, *J* = 1.5 Hz, 1 H)	6.46 (d, *J* = 2.2 Hz, 1 H)
6.11 (dd, *J* = 10.6, 17.6 Hz, 1 H)	6.11 (dd, *J* = 10.3, 17.6 Hz, 1 H)
5.09 (d, *J* = 10.6 Hz, 1 H); 5.09 (d, *J* = 17.6 Hz, 1 H)	5.09 (dd, *J* = 1.2, 10.3 Hz, 1 H) 5.09 (dd, *J* = 1.2, 17.6 Hz, 1 H)
2.27 (s, 3 H)	2.28 (d, *J* = 0.7 Hz, 3 H)
1.53 (s, 3 H)	1.53 (s, 3 H)
1.49 (s, 3 H)	1.49 (s, 3 H)

[5] Wang, W.-L.; Zhu, T.-J.; Tao, H.-W.; Lu, Z.-Y.; Fang, Y.-C.; Gu, Q.-Q.; Zhu, W.-M. *Chem. Biodiv.* **2007**, *4*, 2913–2919.

Table S7. ^{13}C NMR data comparison between reported natural variecolortide B (**1b**) and synthetic variecolortide B (**1b**).

Literature report[5] (^{13}C, 150 MHz, DMSO-d_6)	This report (^{13}C, 150 MHz, DMSO-d_6)
189.4	189.5
165.0	165.2
165.0	164.9
161.4	161.4
161.4	161.3
156.1	156.1
145.1	145.1
145.0	145.0
138.8	138.8
136.3	136.3
136.1	136.2
135.2	135.2
126.4	126.3
126.1	126.0
123.6	123.6
120.9	120.8
119.7	119.8
119.5	119.5
119.5	119.4
119.4	119.4
117.4	117.3
114.7	114.7
111.9	111.8
111.6	111.6
109.0	109.0
107.2	107.4
104.0	103.9

103.7	103.6
103.7	103.4
84.1	84.0
39.4	39.4
27.9	27.9
27.4	27.4
15.9	15.9

Theoretical Calculations - Methods

Geometry optimizations of all species have been performed at the B3LYP/6-31G(d) level of theory. Open-shell species have been treated using an unrestricted wavefunction and all closed-shell species have been described using a restricted wavefunction. Thermal corrections to 298.15 K have been obtained at the same level of theory using the rigid rotor/harmonic oscillator model. Energy values cited in the text refer to enthalpies at 298.15 K and 1 bar pressure in the gas phase. Radical stability values have also been determined with aid of the G3(MP2)-RAD method developed by Radom et al.,[6] and the IMOMO model by Morokuma et al.[7,8] The UCCSD(T)/6-31G(d) calculations required for the G3(MP2)-RAD model have been executed with MOLPRO 2006.1,[9] while all other calculations have been performed with Gaussian 03, Rev. D.01.[10]

Theoretical Calculations - Results

Cycloaddition reaction of quinone **8** with dienophile **18** proceeds in a concerted, asynchonous manner over relatively high reaction barriers to yield the cycloadducts **23** and its diastereomer **23b**. Formation of **23** is endothermic by +55.1 kJ/mol and proceeds over a barrier of +133.7 kJ/mol, while formation of **23b** is endothermic by +60.4 kJ/mol and faces a barrier of +137.9 kJ/mol (all enthalpies computed at B3LYP/6-31G(d) level of theory). Quinone **8** can isomerize through a 1,5-H-shift pathway to tautomer **22**, which is less stable than **8** by +16.7 kJ/mol. Cycloaddition reactions of this less stable tautomer with dienophile **18** are significantly exothermic and yield two stereoisomers **24** and **24b** (Scheme S1). Formation of **24** is exothermic by -49.8 kJ/mol and faces a barrier +74.2 kJ/mol, while formation of **24b** is exothermic by -42.1 kJ/mol with a barrier of +79.3 kJ/mol. With respect to the dramatically different reaction energetics and reaction barriers it is clear that only formation of cycloaddition products **24** and **24b** is to be expected under the selected reaction conditions.

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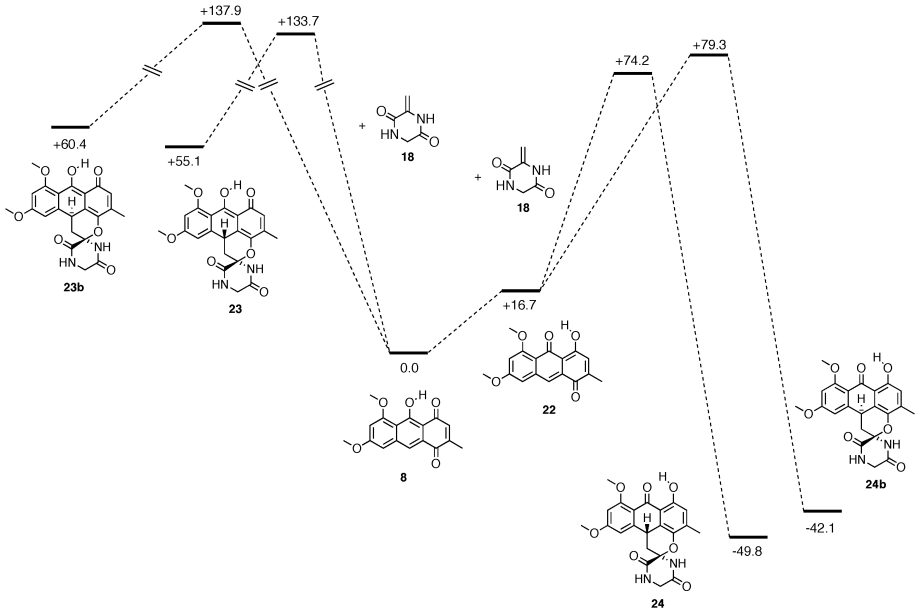
[6] (a) Henry, D. J.; Parkinson, C. J.; Radom, L. *J. Phys. Chem. A* **2002**, *106*, 7927; (b) Henry, D. J.; Sullivan, M. B.; Radom, L. *J. Chem. Phys.* **2003**, *118*, 4849–4860.

[7] (a) Vreven, T.; Morokuma, K. *J. Chem. Phys.* **1999**, *111*, 8799–8803; (b) Vreven, T.; Morokuma , K. *J. Comp. Chem.* **2000**, *21*, 1419–1432.

[8] Coote, M. L.; Lin, C. Y.; Zipse, H. *"The Stability of Carbon-Centered Radicals"*, p. 83 - 104, in M. D. E. Forbes (Ed.), *Carbon-Centered Free Radicals and Radicals Cations*, Wiley, John Wiley & Sons, **2010**.

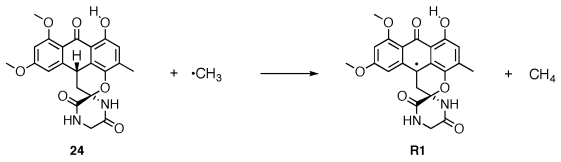
[9] MOLPRO, version 2006.1, a package of *ab initio* programs, H.-J. Werner, P. J. Knowles, R. Lindh, F. R. Manby, M. Schütz, and others, see www.molpro.net.

[10] Gaussian 03, Revision D.01, **2004**. The full citation is available at www.gaussian.com/citation_g03.htm.



Scheme S1

The cycloadducts **24** and **24b** appear to contain a rather weak C-H bond at one of the former reaction centers. The radical stabilization energy (RSE) of the resulting radical **R1** can be quantified relative to methyl radical •CH_3 using the isodesmic reaction shown in Scheme S2. The corresponding strength of the C-H bond in cycloadduct **24** can then be calculated from the RSE value and the known C-H BDE of methane of BDE(H_3C-H) = +439.3 kJ/mol.[11] Radical stabilization energies (RSE) for a number of related systems have also been calculated and are compiled in Table S8.

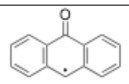
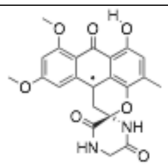


Scheme S2

[11] Luo, Y.-R. *Comprehensive Handbook of Chemical Bond Energies*, CRC Press, **2007**.

The benzhydryl radical •$CH(Ph)_2$ is one of the best studied and structurally closely related systems. This radical is 85.5 kJ/mol more stable than methyl radical •CH_3 and the bond strength in the respective closed shell system thus corresponds to +439.3 - 85.5 = +353.8 kJ/mol. The effects of the bridging C=O-unit in radical **R1** can be quantified by calculating the stability of model radical **R2**. The RSE value of -100.1 kJ/mol for this system indicates that introduction of this bridging unit reduces the C-H bond strength to BDE(C-H, **R2**) = +339.2 kJ/mol.

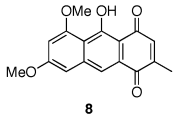
Table S8. Radical stabilization energies (RSE) and bond dissociation energies (BDE) of selected systems. Radicals are drawn with the unpaired electron at the location of the former C-H bond.

System	RSE [kJ/mol]	BDE(calc) [kJ/mol]	BDE(exp.) [kJ/mol]	ref.
•CH_3	0.0	-	+439.3±0.4	11
•$CH(Ph)_2$	-85.5 (G3(MP2)-RAD)	+353.8	+353.5±2.1	11,8
 R2	-100.1 (G3B3)	+339.2		
 R3	-119.5 (G3)	+319.8	+318.0±5.0	11,12
 R1	-123.4 (IMOMO(G3B3:B3LYP))	+315.9		

Considering all further details of the molecular decoration present in **R1** as a correction to the BDE value calculated for **R2** using the IMOMO scheme finally predicts a BDE(C-H,**R1**) = +315.9 kJ/mol. This puts the C-H bond strength in cycloaddition product **24** in close proximity to those in known reductants such as 1,4-cyclohexadiene (yielding radical **R3**) with BDE(C-H) = +319.8 kJ/mol or thiophenol with BDE(S-H) = +335.4 kJ/mol.[13]

[12] Feng, Y.; Liu, L.; Wang, J.-T.; Huang, H.; Guo, Q.-X. *J. Chem. Inf. Comput. Sci.* **2003,** *43,* 2005–2013.
[13] Hioe, J.; Zipse, H. *Org. Biomol. Chem.* **2010**, *8*, 3609–3617.

NMR spectra.



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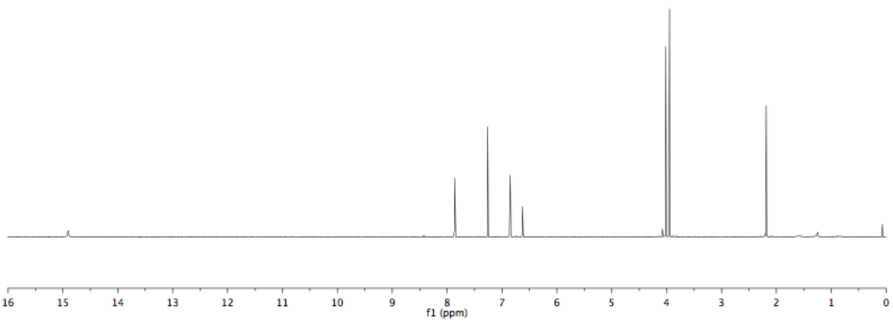

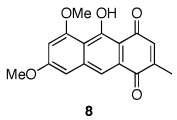

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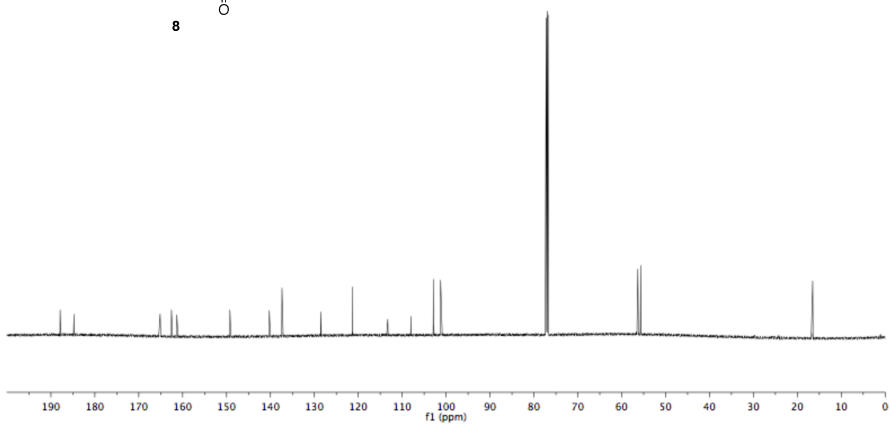
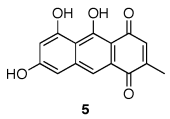
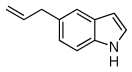
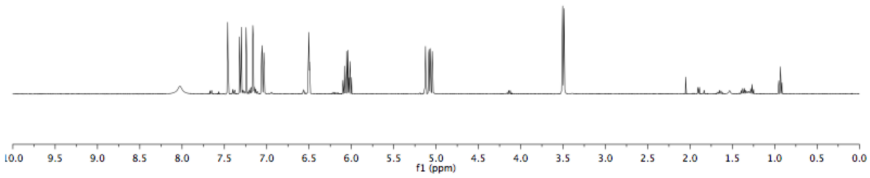




5



10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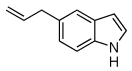
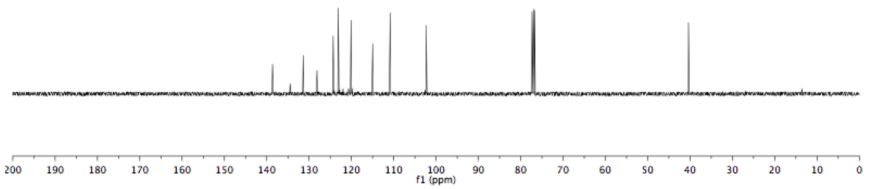
f1 (ppm)



10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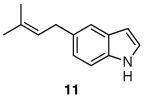
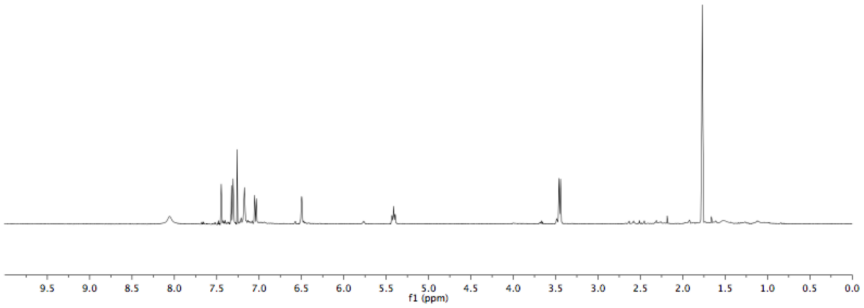
f1 (ppm)


N
H

11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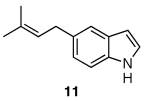
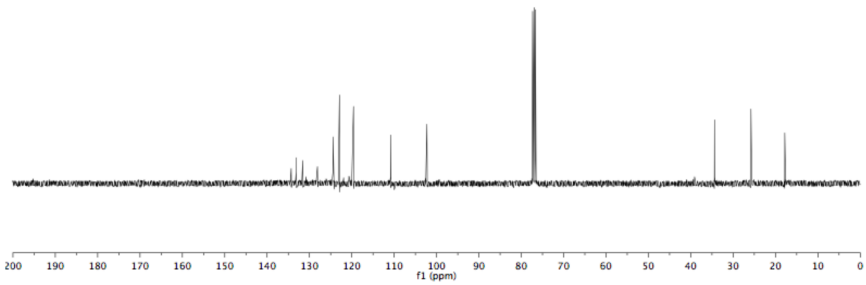
f1 (ppm)


N
H

11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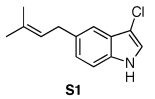
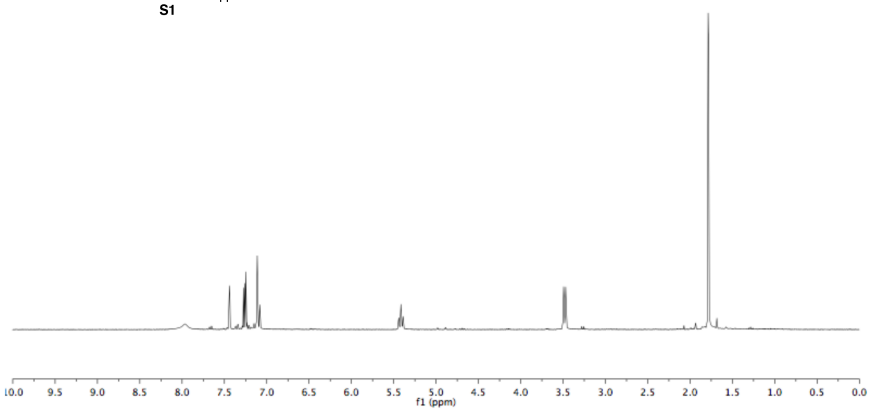
f1 (ppm)


Cl
N
H

S1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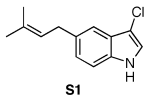
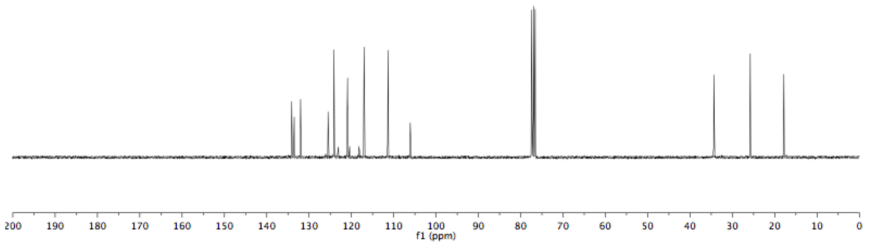
f1 (ppm)


Cl
N
H

S1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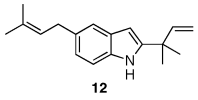
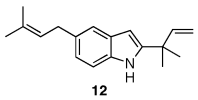
f1 (ppm)

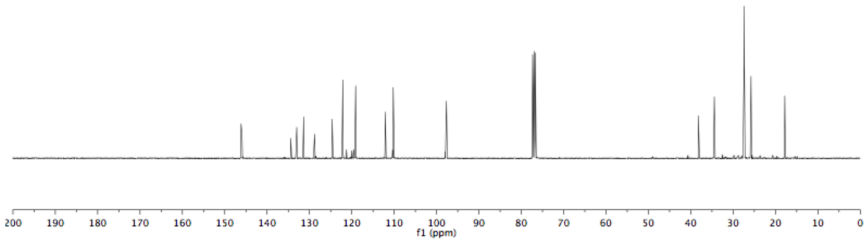

N
H

12

10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)


N
H

12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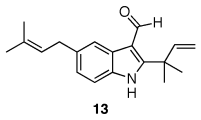
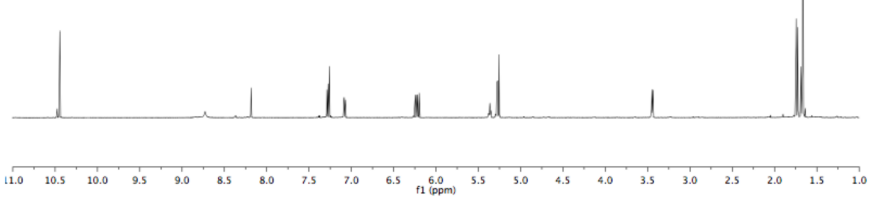
f1 (ppm)


O
H
N
H

13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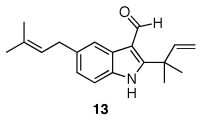
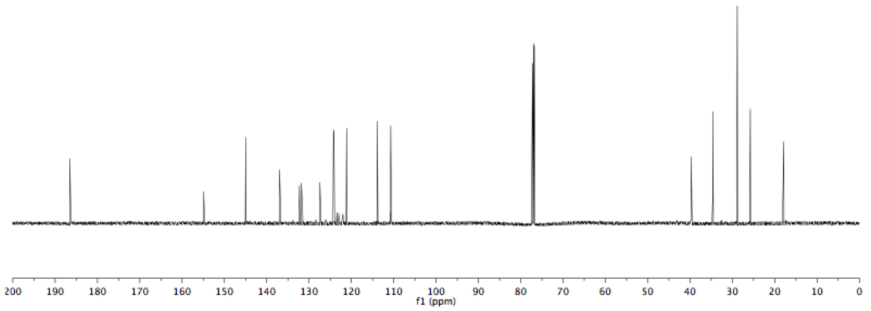
f1 (ppm)


O
H
N
H

13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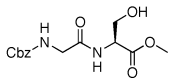
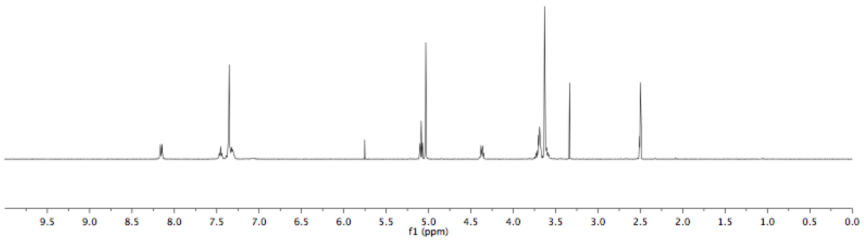
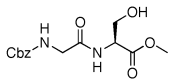
f1 (ppm)

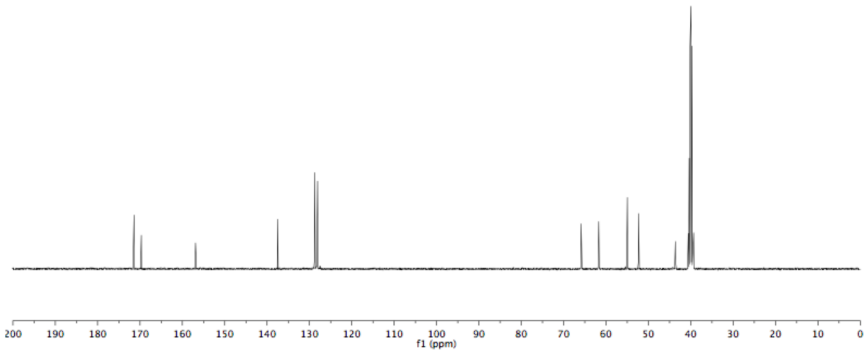

OH
H
N
Cbz
O
N
H
O
O

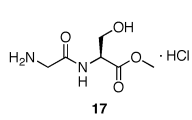
16


9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)


OH
H
N
Cbz
O
N
H
O
O

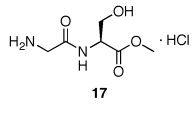
16


200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

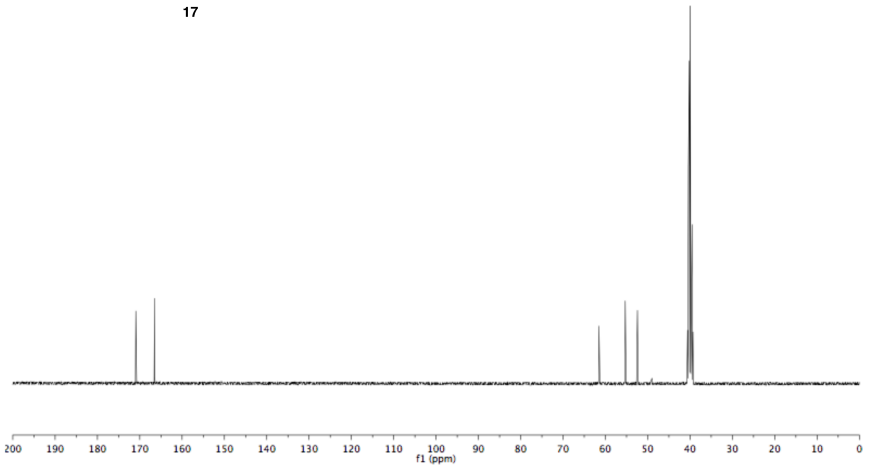

OH
O
H2N
N
H
O
O
· HCl

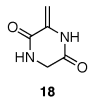
17

9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
f1 (ppm)


OH
O
H2N
N
H
O
O
· HCl

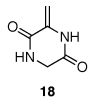
17


200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

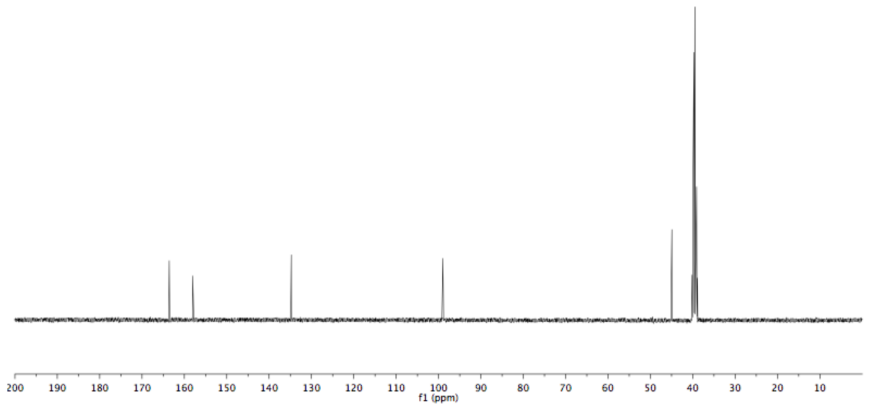

O
NH
HN
O

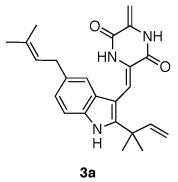
18

11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)


O
NH
HN
O

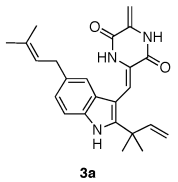
18


200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10
f1 (ppm)


O
NH
HN
O
N
H

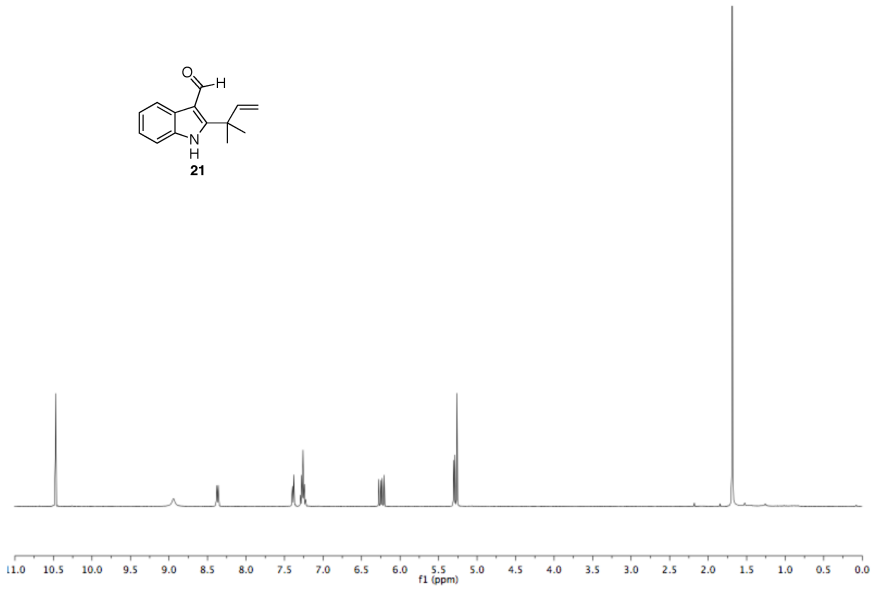
3a

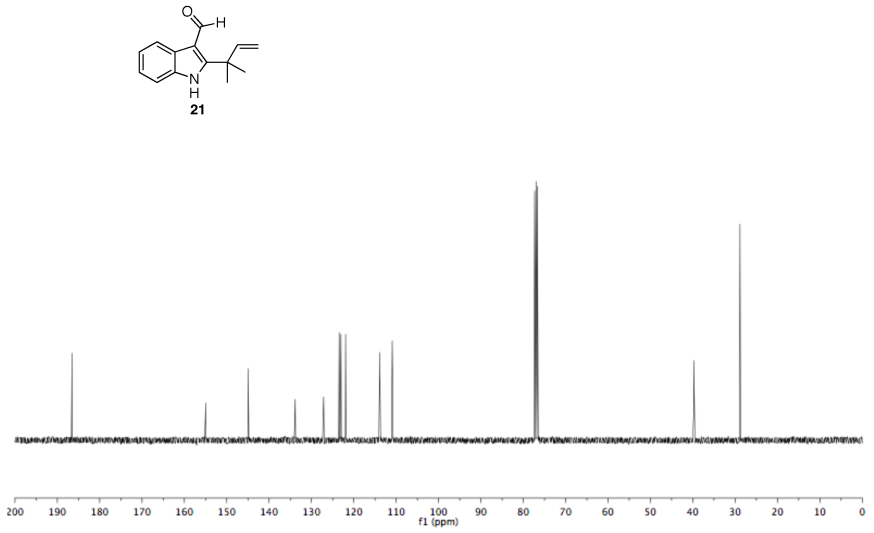
10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)

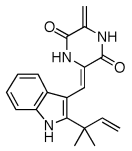

O
NH
HN
O
N
H

3a

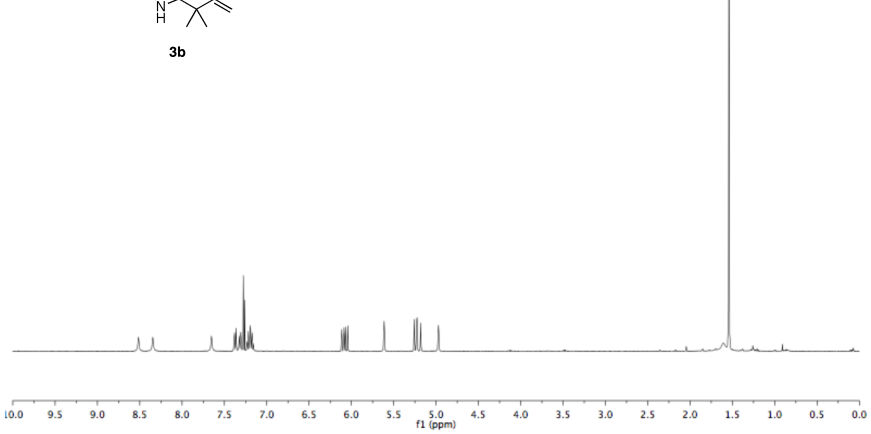
200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

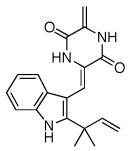

O
H
N
H
21
11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)


O
H
N
H
21
200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)


O
NH
HN
O
N
H

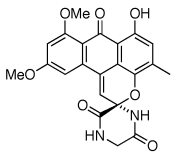
3b


10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)

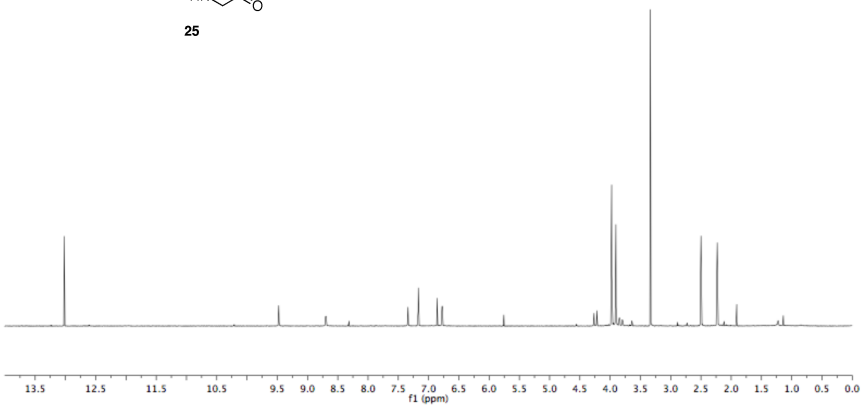

O
NH
HN
O
N
H

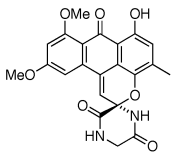
3b

200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)


OMe
O
OH
MeO
O
O
NH
HN
O

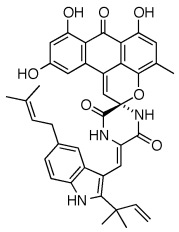
25


13.5 12.5 11.5 10.5 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)


OMe
O
OH
MeO
O
O
NH
HN
O

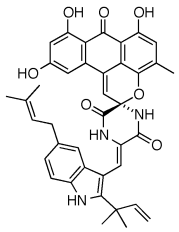
25

200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)



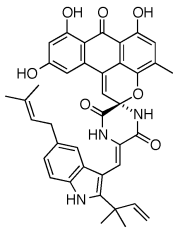
variecolortide A (**1a**)

after purification by column chromatography



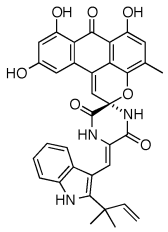
variecolortide A (**1a**)

after purification by column chromatography



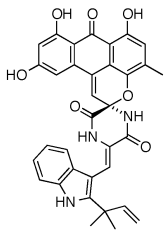
variecolortide A (**1a**)

after purification by HPLC



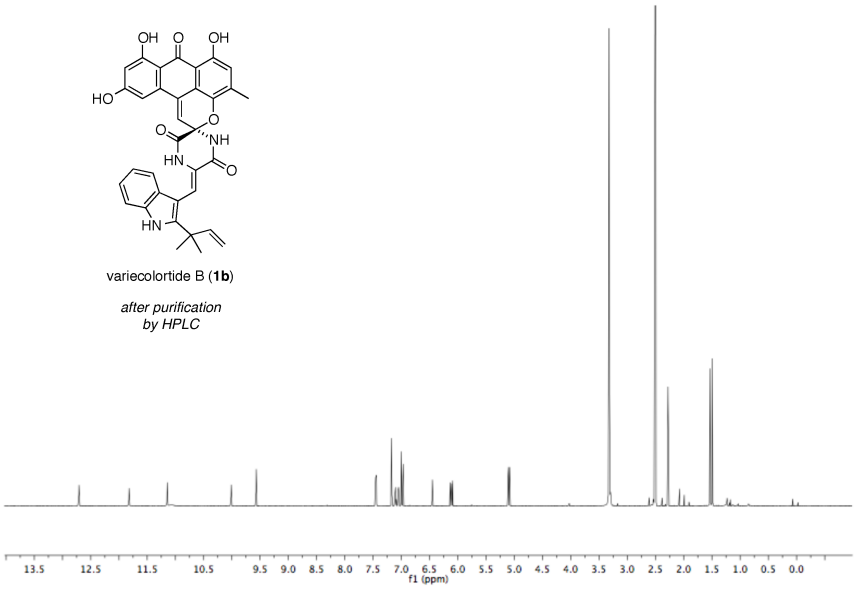
variecolortide B (**1b**)

after purification by column chromatography



variecolortide B (**1b**)

after purification by column chromatography



variecolortide B (**1b**)

after purification by HPLC

Zuschriften

Naturstoffsynthese

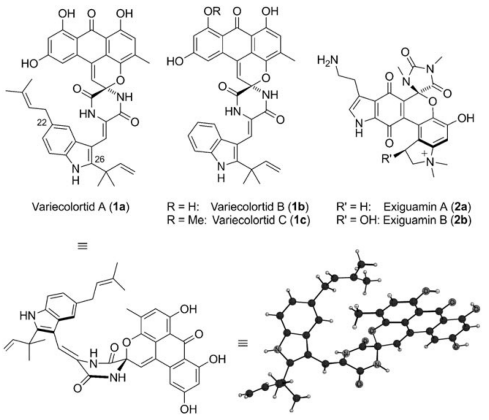
DOI: 10.1002/ange.201006154

Kompakte Totalsynthese von Variecolortid A und B durch eine ungewöhnliche Hetero-Diels-Alder-Reaktion**

Christian A. Kuttruff, Hendrik Zipse und Dirk Trauner**

Seit jeher werden Synthesechemiker durch Spekulationen über die Biosynthese von Naturstoffen inspiriert, was bereits zu vielen eleganten und effizienten Totalsynthesen geführt hat.[1] Viele dieser Totalsynthesen enthalten Kaskadenreaktionen, in denen ein energetisch angeregtes Substrat gebildet wird, das anschließend spontan zu einem deutlich komplexeren Molekül weiterreagiert.[2] Solche Kaskadenreaktionen sind oft Teil einer biomimetischen Strategie und kommen ohne die Verwendung von Enzymen aus, die an der echten Biosynthese beteiligt sind.

Vor kurzem publizierten wir die Synthese der vom Dopamin abgeleiteten Alkaloide Exiguamin A und B (**2a, b**; Schema 1), die auf einer biomimetischen Kaskade aus peri-



Schema 1. Racemische Naturstoffe mit N,O-Acetal.

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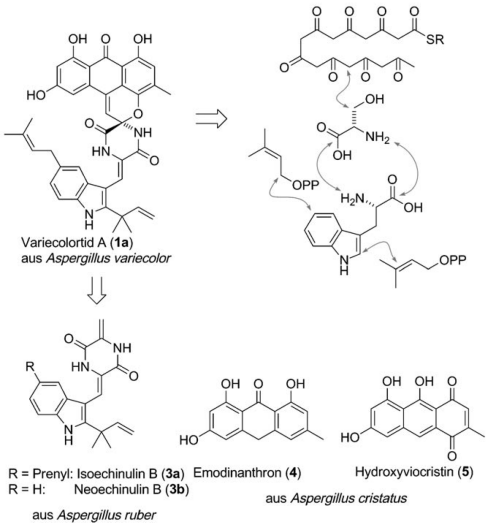
cyclischer Reaktion und Oxidation beruht.[3] In der Natur kommen die Exiguamine als Racemate vor und enthalten als Schlüsselelement ein spirobicyclisches N,O-Acetal. Die Variecolortide (**1a–c**) haben daher als kürzlich entdeckte Familie racemischer N,O-Acetale unser Interesse geweckt.[4] Diese ungewöhnlichen Naturstoffe wurden aus einem halotoleranten Stamm des Pilzes *Aspergillus variecolor* isoliert und haben schwach cytotoxische Eigenschaften.[4]

Strukturell betrachtet weisen die Variecolortide ein bisher einzigartiges 9,10-Anthrachinonmethid-Gerüst auf, das an einen Dihydropyran-Ring gebunden ist (Schema 1). Das flache tetracyclische Ringsystem ist über ein N,O-Acetal mit einem Diketopiperazin verbunden. Des Weiteren ist das zentrale Diketopiperazin mit einem Indol-Rest verknüpft, der an Position C26 revers prenyliert ist. Variecolortid A (**1a**) ist wegen der zusätzlichen Prenyl-Gruppe an Position C22, die bei Variecolortid B (**1b**) und dessen Methylether Variecolortid C (**1c**) fehlt, das komplexeste Mitglied der Familie.

Aus Biosynthesesicht scheinen die Variecolortide von einem C16-Polyketid, den Aminosäuren Serin und Tryptophan sowie ein oder zwei Äquivalenten Dimethylallylpyrophosphat abzustammen (Schema 2). Damit repräsentieren die Variecolortide eine einzigartige Kombination dreier

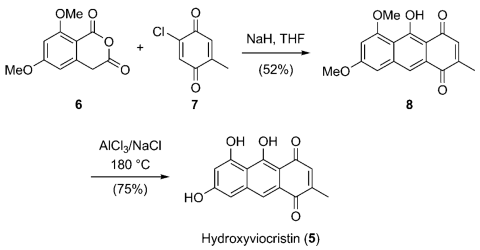


Schema 2. Biosynthetische Analyse der Variecolortide. PP = Diphosphat.

Hauptbiosynthesewege: des Shikimisäure-Wegs (für die aromatische Aminosäure Tryptophan), des Typ-II-Polyketid-Wegs (für die Anthrachinon-Derivate) und des Terpenoid-Wegs (für die Prenyl- und die inverse Prenyl-Seitenkette). Zwar kennt man viele Naturstoffe, die drei oder mehr verschiedene Biosynthesewege vereinen, allerdings ist genau diese Kombination selten, wenn nicht sogar unbekannt.

Einige der Strukturelemente der Variecolortide sind selbst als Naturstoffe bekannt. Bereits vor einigen Jahrzehnten publizierten Laatsch und Anke die Strukturen von Emodinanthron (**4**) und Hydroxyviocristin (**5**), einem Anthron bzw. 1,4-Anthrachinon, die Substitutionsmuster entsprechend der Variecolortide aufweisen (Schema 2).[5] Diese Verbindungen wurden aus *Aspergillus cristatus* isoliert. Das ungesättigte Diketopiperazin Isoechinulin B (**3a**)[6] wurde zusammen mit weiteren Abkömmlingen, denen eine Prenyl-Gruppe fehlt oder bei denen die Exomethylen-Gruppe oxidativ gespalten, hydriert oder als Methanol-Addukt maskiert ist, aus *Aspergillus ruber* isoliert.[7] Zu Beginn unserer Studien war unklar, auf welche Weise diese Naturstoffe, über deren Vorkommen in *Aspergillus variecolor* noch nichts bekannt ist, kombiniert werden, um die Variecolortide zu bilden. Wir berichten nun über eine effiziente Totalsynthese von **1a** und **1b**, die eine überraschend einfache Lösung für die Verknüpfung des Anthrachinon- und Diketopiperazin-Bausteins bietet und dabei auf einer neuartigen Diels-Alder-Reaktion[8] aufbaut, die auch relevant für die Biosynthese sein könnte.

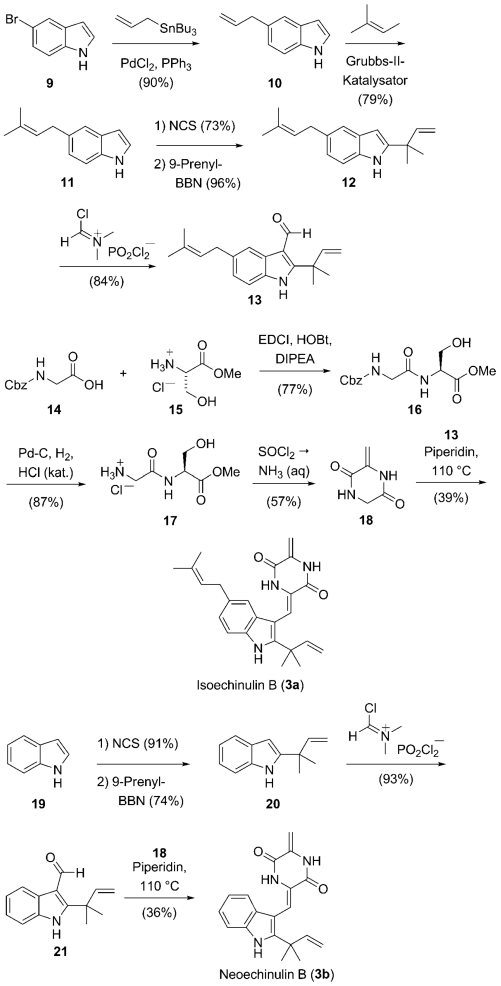
Unsere Totalsynthese der Variecolortide begann mit der Herstellung von Hydroxyviocristin (**5**; Schema 3). Deprotonierung des bekannten, von der Orsellinsäure abgeleiteteten



Schema 3. Totalsynthese von Hydroxyviocristin (**5**).

Anhydrids **6**,[9] gefolgt von einer Addition des resultierenden benzylischen Anions in Chlor-*para*-benzochinon **7**, führte zur Bildung des bekannten 1,4-Anthrachinons **8**[10] mithilfe einer konjugierten Additions-Decarboxylierungs-Sequenz. Anschließende Demethylierung in geschmolzenem $AlCl_3$/NaCl ergab Hydroxyviocristin (**5**).

Die Synthese des Diketopiperazin-Indol-Bausteins startete mit einer Palladium-katalysierten Kreuzkupplung von 5-Bromindol (**9**) mit Allyltributylstannan, gefolgt von einer Grubbs-Olefinkreuzmetathese, um das prenylierte Indol **11** zu erhalten (Schema 4). Die Einführung der inversen Prenyl-Gruppe erfolgte durch Umsetzung von **11** unter Danishefsky-Bedingungen zu **12**.[11] Formylierung von **12** mit dem Vilsmeier-Reagens führte zum Aldehyd **13**. Zum Einbau des

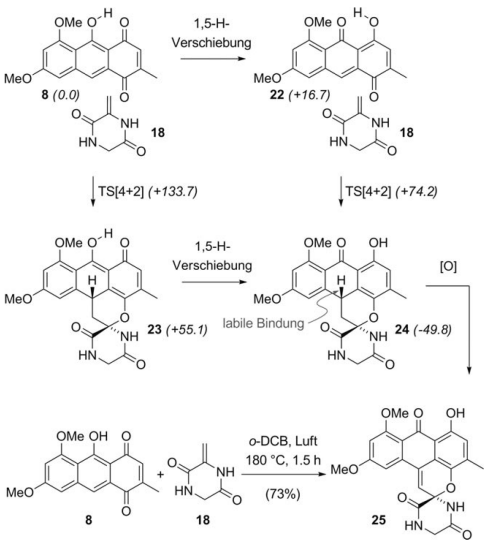


Schema 4. Totalsynthesen von Isoechinulin B (**3a**) und Neoechinulin B (**3b**). BBN = 9-Borabicyclo[3.3.1]nonan, Cbz = Benzyloxycarbonyl, DIPEA = *N*,*N*-Diisopropylethylamin, EDCI = *N*′-(3-Dimethylaminopropyl)-*N*-ethylcarbodiimid, NCS = *N*-Chlorsuccinimid, HOBt = 1-Hydroxybenzotriazol.

Aminosäure-Bausteins wurde *N*-Cbz-Glycin (**14**) mit Serinmethylester (**15**) zum Dipeptid **16** kondensiert, das nach Entschützung der Cbz-Gruppe, Cyclisierung und formaler Eliminierung von Wasser Exomethylen-Diketopiperazin **18** ergab. Kondensation von **18** mit Formylindol **13** führte nach einer Methode von Kishi et al. zu Isoechinulin B (**3a**), das als einziges Isomer erhalten wurde.[12] Eine analoge Sequenz ausgehend von Indol (**19**→**20**→**21**) führte zu Neoechinulin B (**3b**; Schema 4).

Zuschriften

Mit ausreichenden Mengen an Hydroxyviocristin (**5**) und den Echinulinen (**3a**,**b**) zur Verfügung, untersuchten wir die entscheidende Verknüpfung beider Komponenten. Ursprünglich planten wir die Verwendung nucleophiler oder radikalischer Additionen mit **5** oder Emodinanthron (**4**) als Polyketid-Baustein. Diese Strategie wurde jedoch wegen der schwachen Reaktivität der Exomethylen-Diketopiperazine gegen anionische Nucleophile und der unzureichenden Kontrollierbarkeit von Radikaladditionen an Emodinanthron verworfen. Wir wandten uns deshalb einer Cycloadditionsstrategie zu, die zunächst anhand eines Modellsystems sowohl experimentell als auch computerbasiert erprobt wurde (Schema 5). Wir nahmen an, dass bei Hydroxyviocristin (**5**)



Schema 5. Modellsystem für die Schlüsselcycloaddition. Die relativen Energien der Schlüsselintermediate und die Aktivierungsbarrieren sind in Klammern angegeben (siehe Text). *o*-DCB = *ortho*-Dichlorbenzol, TS[4+2] = Übergangszustand der [4+2]-Cycloaddition.

und dessen Dimethylether **8** eine intramolekulare 1,5-H-Verschiebung zum Tautomer **22** stattfinden könnte. Dieses reaktive Intermediat wäre in der Lage, in einer Hetero-Diels-Alder-Reaktion mit Exomethylen-Diketopiperazin **18** zum Spiro-N,O-acetal **24** zu reagieren. Ein solches Anthron-Intermediat weist eine äußerst labile benzylische C-H-Bindung auf und würde somit an Luft leicht zum 9,10-Anthrachinonmethid **25** oxidiert werden. In der Tat konnte **25** als einziges Produkt isoliert werden, wenn **8** und **18** in einem Druckrohr unter aeroben Bedingungen erhitzt wurden (Schema 5). Diese Verbindung entspricht dem oberen Teil der Variecolortide und käme sogar als Intermediat in deren Synthese in Frage.

Der von uns vorgeschlagene Diels-Alder-/Oxidations-Mechanismus wird durch Dichtefunktionalrechnungen auf B3LYP/6-31G(d)-Niveau gestützt (siehe Schema 5 und Abbildung S1 in den Hintergrundinformationen). Entsprechend dieser Rechnungen reagiert Tautomer **8** mit **18** über einen konzertierten, asynchronen Pfad zum Cycloadditionsprodukt **23**. Dieser Prozess ist deutlich endotherm und hat mit $+133.7\ kJ\,mol^{-1}$ eine relativ große Reaktionsbarriere. Im Vergleich dazu ist Tautomer **22** um $16.7\ kJ\,mol^{-1}$ instabiler als **8**, dafür aber deutlich reaktiver in Bezug auf die Diels-Alder-Reaktion mit **18**. Die Reaktionsbarriere beträgt nun $+74.2\ kJ\,mol^{-1}$, und die Bildung des Cycloadditionsprodukts **24** ist mit $49.8\ kJ\,mol^{-1}$ exotherm. Angesichts dieser beachtlichen Unterschiede bei den Reaktionsenergien und -barrieren ist klar, dass nur die Cycloaddition über Tautomer **22** unter den experimentellen Bedingungen relevant ist.

Die berechnete Geometrie des Übergangszustands dieser Reaktion ist in Abbildung 1 dargestellt. Unseren Rechnungen zufolge fungiert das Exomethylen-Diketopiperazin **18** als die nucleophile Komponente in der asynchronen Cycloaddition, wobei im Übergangszustand die Bildung der C-C-Bindung weiter fortgeschritten ist als die der C-O-Bindung.

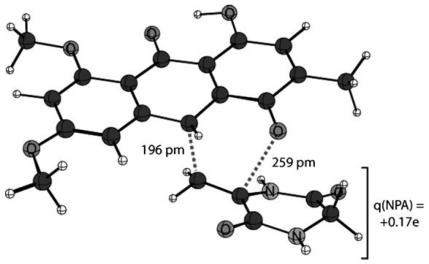
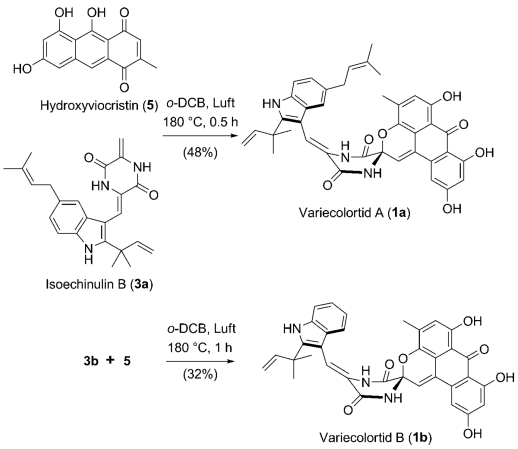


Abbildung 1. Berechneter Übergangszustand der asynchronen Hetero-Diels-Alder-Reaktion von **22** mit **18**. Die Längen der sich bildenden Bindungen und die partielle Ladung *q*(NPA) (NPA = natural population analysis) des Heterodienophils sind angedeutet.

Um die Plausibilität des vorgeschlagenen Oxidationsprozesses von **24** zum isolierten Reaktionsprodukt **25** zu überprüfen, wurde die Stabilität der bisbenzylischen C-H-Bindung (siehe Abbildung 1) mithilfe einer Serie von Referenzverbindungen auf G3B3-Niveau berechnet. Durch Verwendung geeigneter isodesmischer Reaktionen lässt sich die Bindungsenergie (BDE) dieser C-H-Bindung auf $+315.9\ kJ\,mol^{-1}$ abschätzen (für Details siehe Hintergrundinformationen). Dieser Wert ist damit sogar geringer als die entsprechenden Werte für gängige Reduktionsmittel, wie 1,4-Cyclohexadien [BDE(C-H) = $+318.0\ kJ\,mol^{-1}$], $HSnBu_3$ [BDE(Sn-H) = $+328.9\ kJ\,mol^{-1}$] oder Thiophenol [BDE(S-H) = $+335.4\ kJ\,mol^{-1}$] und stützt deshalb vollständig den von uns vorgeschlagenen In-situ-Oxidationspfad.[13]

Auf Grundlage der gewonnenen Einblicke und des anhand der Modellverbindungen optimierten Schlüsselschrittes konnten wir uns der Fertigstellung der Totalsynthese der Variecolortide zuwenden (Schema 6). Wir erwarteten, dass innerhalb der Echinuline die disubstituierte Exomethylen-Einheit das reaktivste Heterodienophil sein und dass das



Schema 6. Totalsynthese von Variecolortid A und B.

korrekte Regioisomer gebildet werden würde. Tatsächlich entstand beim Erhitzen von Hydroxyviocristin (**5**) und Isoechinulin B (**3a**) in *ortho*-Dichlorbenzol Variecolortid A in 48% Ausbeute. Unter den gleichen Bedingungen konnte Variecolortid B aus den Bausteinen **5** und **3b** erhalten werden. Die moderaten Ausbeuten der Schlüsselreaktionen sind vermutlich auf die bekannte thermische Instabilität von Hydroxyviocristin zurückzuführen.[5] Die Variecolortide waren die einzigen Isomere, die wir unter diesen Bedingungen isolieren konnten; weder Regioisomere noch Nebenreaktionen anderer Doppelbindungen der Echinuline wurden beobachtet.

Die verwendeten Bedingungen in unserer Schlüsselreaktion können zweifellos nicht als „biomimetisch“ erachtet werden, angesichts der konzertierten Abfolge stellen sich jedoch einige interessante Fragen zur Biosynthese. In orientierenden Experimenten konnte unter biomimetischeren Bedingungen (wässriger Phosphatpuffer bei Raumtemperatur) bisher kein Produkt isoliert werden. Angesichts dieser Befunde und den berechneten Aktivierungsbarrieren scheinen die Variecolortide keine Produkte einer zufälligen, unkatalysierten Diels-Alder-Reaktion innerhalb des Pilzes zu sein. Dies wirft die Frage auf, ob eine „Diels-Alderase“ involviert sein könnte.[14] Zwar ist es durchaus denkbar, dass **5** und **3a**/**3b** echte Biosynthesevorstufen der Variecolortide sind, allerdings darf die Möglichkeit einer Verknüpfung der beiden Bausteine vor Freisetzen des Polyketid-Rests von der Typ-II-Polyketidsynthase nicht außer Acht gelassen werden. Die genaue Abfolge, in der die einzelnen Biosynthesekomponenten der Variecolortide zusammenkommen, und der Mechanismus ihrer Verknüpfung bleiben noch zu klären.

Wir haben eine kompakte Totalsynthese der Variecolortide A und B entwickelt, die die natürlichen Racemate in sieben bzw. fünf Stufen (längste lineare Sequenz) zugänglich macht. Unsere Synthese ist beinahe schutzgruppenfrei[15] und hoch konvergent. Sie umfasst eine neuartige Hetero-Diels-Alder-Reaktion eines 1,4-Anthrachinons mit einem Didehydrodiketopiperazin, um den zentralen spirocyclischen Kern der Naturstoffe zu bilden. Dichtefunktionalrechnungen stützen unsere Hypothese, dass die Schlüsselreaktion über eine konzertierte Cycloaddition verläuft. Unsere Synthese wirft die Frage auf, ob nicht ähnliche Reaktionen in der Natur unter Beteiligung eines entsprechenden Enzyms ablaufen könnten. Die Beantwortung dieser Frage erfordert jedoch detaillierte Biosynthesestudien, die den Rahmen dieser Zuschrift sprengen würden. Auf jeden Fall bietet unsere Laborsynthese einen effizienten Zugang zu den Variecolortiden und ermöglicht damit eine ausgiebige biologische Evaluierung dieser faszinierenden Naturstoffe.

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Stichwörter: Alkaloide · Biomimetische Synthesen · Cycloadditionen · Kaskadenreaktionen · Totalsynthesen

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2.1.2 Evolution of a Synthetic Strategy for the Variecolortides

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FULL PAPER

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Evolution of a Synthetic Strategy for the Variecolortides

Christian A. Kuttruff,[a] Peter Mayer,[a] and Dirk Trauner*[a]

Dedicated to the memory of Ernesto Fattorusso

Keywords: Total synthesis / Natural products / Cycloaddition / Biomimetic synthesis / Domino reactions / Cascade reactions

The variecolortides are a family of unusual natural products that combine motifs from a variety of biosynthetic streams. Herein, we present the gradual evolution of a convergent synthetic strategy that ultimately culminated in a reaction cascade featuring a hydrogen shift and a cycloaddition followed by a spontaneous air oxidation. Attempts to link an anthrone building block with an *exo*-methylene diketopiperazine using radical chemistry were ultimately unsuccessful, but led to interesting observations that shaped our successful strategy. The total synthesis of variecolortide C is presented for the first time.

Introduction

Nature remains a major source of inspiration for synthetic chemists not only in terms of the structures it produces but also with respect to the strategies it uses for their construction. These have been mimicked with remarkable success in the laboratory, yielding many elegant and efficient syntheses of natural products.[1] Many of these "biomimetic" syntheses incorporate reaction cascades, whose role in the origin of natural products has been increasingly recognized. Cascade reactions allow for the formation of many bonds in a single operation and can create several stereocenters, which also allows for the spontaneous construction of complex chiral molecules from achiral precursors.[2] However, in the absence of an asymmetric environment, such as the active site of an enzyme, there is no reason why one enantiomer should be preferred over the other. Hence, complex natural products that have been isolated in racemic form can be suspected to spontaneously arise through cascade reactions and, thus, are prime candidates for biomimetic total synthesis.

In recent years, our group has published several syntheses of racemic natural products, including rubicordifolin (**1**),[3] rubioncolin B (**2**),[4] and exiguamines A (**3**) and B (**4**)[5] (see Figure 1) that were based on pericyclic reaction cascades. More recently, the variecolortides attracted our attention. These unusual compounds were isolated from the halotolerant fungal strain *Aspergillus variecolor* B-17, and their structures and biological activities were reported by Wang et al. in 2007.[6] They feature a tetracyclic pyrano-anthrone moiety, which is connected to a central diketopiperazine

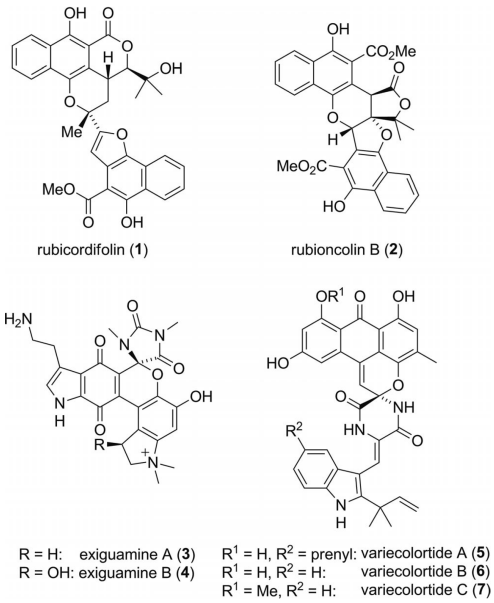

Figure 1. Racemic natural products that have been synthesized in our laboratory.

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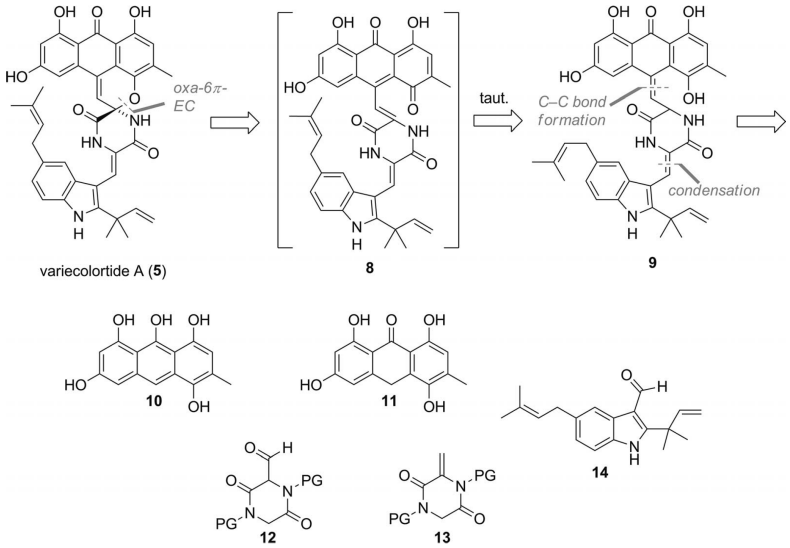
moiety through a spirocyclic *N*,*O*-acetal. Notably, this structural motif can also be found in the exiguamines. The diketopiperazine moiety of the variecolortides is also connected to a reversely prenylated indole, which can be further prenylated on the benzene ring. Variecolortide A (**5**), B (**6**), and C (**7**) are distinguished by different prenylation patterns (prenyl = 3-methyl-but-2-en-1-yl) as well as differences in the *O*-methylation of the pyrano-anthrone unit.

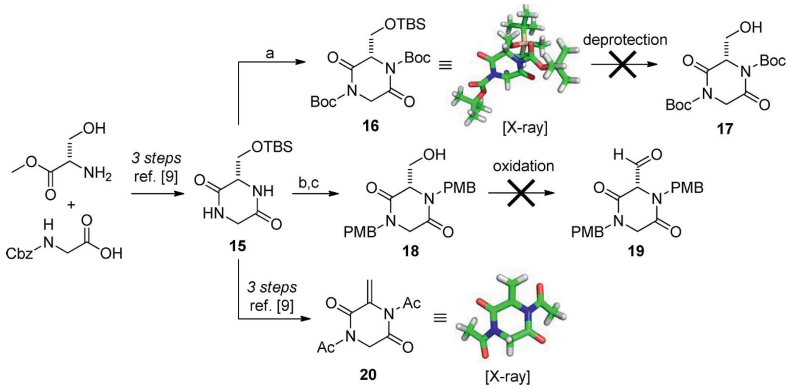
In a recent communication, our group published the syntheses of variecolortide A and B that hinged once again on a pericyclic reaction cascade.[7] However, our eventual successful synthetic strategy was not our initial choice, but gradually evolved in the course of our investigations. We now disclose the full details of our studies that eventually led to the total syntheses of variecolortide A and B and report our previously unpublished synthesis of variecolortide C.

Results and Discussion

Our initial plan for the synthesis of the variecolortides is depicted in Scheme 1. We reasoned that the spirobicyclic *N*,*O*-acetal moiety, which also represents the sole stereogenic center of the racemic molecules, could be formed by means of an oxa-6π-electrocyclization (oxa-6π-EC) reac-



Scheme 1. First-generation retrosynthesis of variecolortide A (**5**).



Scheme 2. Syntheses of different diketopiperazines. Reagents and conditions: (a) Boc_2O, DMAP [4-(dimethylamino)pyridine], DMF (dimethylformamide), room temp., 1.5 h, 69%; (b) NaH, PMBCl, 0 °C to room temp., 12 h, 53%; (c) NH_4F, MeOH, 40 °C, 5 d, 99%.

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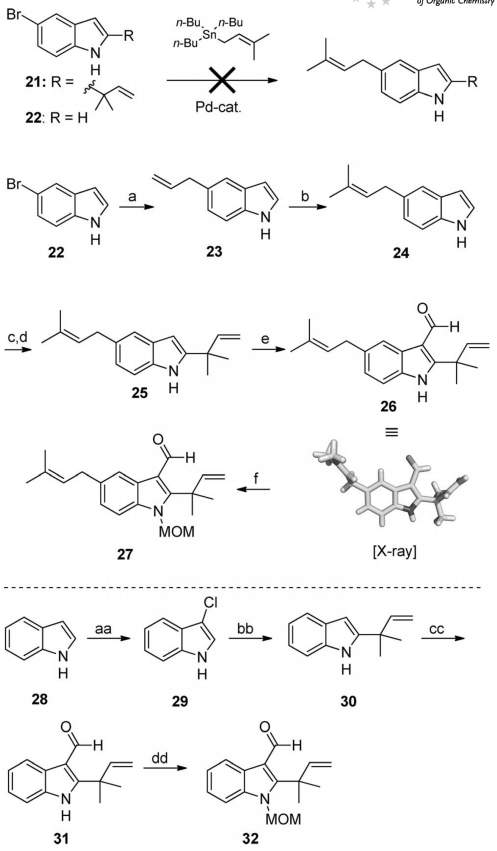
tion. This type of reaction has been successfully used in other total syntheses published by our group, for instance, in the synthesis of exiguamine A (**3**) and B (**4**) and microphyllaquinone.[8] In the retrosynthetic sense, variecolortide A (**5**) would arise from the corresponding hydroquinone **8**, which would stem from anthraquinone methide **9** through a tautomerization (see Scheme 1). The key intermediate **9** could be further dissected into a "northern" anthracene portion (i.e., **10**) or an analogous anthrone moiety (i.e., **11**), a "central" diketopiperazine building block (i.e., **12** or **13**), and a "southern" indole building block **14**.

Our initial approach for the connection of the northern and central part of the variecolortides called for a condensation reaction between the formyl diketopiperazine **12** and the anthracene **10** or anthrone **11**. As we did not find literature precedence for formyl diketopiperazines of type **12**, we planned to synthesize them from their corresponding serine-derived diketopiperazine precursors by simple oxidation (see Scheme 2). To this end, silyl ether **15** was first prepared in three steps from protected amino acids, according to a procedure published by Chai et al.[9] The former was then Boc-protected to give **16**, whose structure and absolute stereochemistry were unambiguously assigned by X-ray crystal structure analysis. However, subsequent attempts to remove the silyl protecting group and oxidize to the resulting alcohol **17** failed. We reasoned that the Boc protecting groups might not survive the deprotection conditions and, therefore, switched to the more stable PMB (*p*-methoxybenzyl) protecting group. Thus, **15** was doubly alkylated to give the corresponding bis(PMB) derivative,[10] which could be desilylated by using ammonium fluoride in methanol. With the desired alcohol **18** in hand, we next screened a variety of different oxidation conditions, again with no success.

The failure to obtain aldehydes of type **12** left us to reconsider our approach, opting instead for electrophiles other than a carbonyl group. As the hydroxymethyl group present in **18** can easily be converted into an *exo*-methylene group, we decided to synthesize a diketopiperazine that could potentially function as a Michael acceptor. To this end, piperazinedione **15** was converted into *exo*-methylene diketopiperazine **20** in three steps, following a known literature procedure.[9] The structure of this compound was also confirmed by X-ray crystal structure analysis.

Next, the synthesis of the southern indole portion was addressed. As variecolortide A is the only member of the family whose indole moiety bears an additional prenyl group at C-22, we had to devise a strategy for this building block (see Scheme 3). Originally, we planned to synthesize **26** from 5-bromoindole derivative **21** by direct prenylation and subsequent formylation. Although direct prenylations of arenes using Stille couplings have been reported,[11] the direct coupling of a prenyl stannane to **21** failed in our hands. We reasoned that the double bond of the reverse-prenyl group present in **21** might interfere with the transition-metal catalysts and thus attempted to couple the prenyl stannane to unsubstituted 5-bromoindole (**22**), but again our efforts were unsuccessful.



Scheme 3. Syntheses of formyl indoles. Reagents and conditions: (a) allyl(tributyl)stannane, $PdCl_2$, PPh_3, DMF, 110 °C, 24 h, 90%; (b) 2-methyl-2-butene, Grubbs second-generation catalyst, CH_2Cl_2, room temp., 1.5 h, 79%, (c) NCS (*N*-chlorosuccinimide), DMF, room temp., 3.5 h, 73%; (d) prenyl-9-BBN (BBN = 9-borabicyclo[3.3.1]nonane), Et_3N, THF (tetrahydrofuran), room temp., 3.5 h, 96%; (e) Vilsmeier reagent, DMF, room temp., 3 h; then NaOH, 20 min, 84%; (f) NaH, MOMCl (methoxymethyl chloride), DMF, 0 °C to room temp., 20 h, 86%; (aa) NCS, DMF, room temp., 1 h, 91%; (bb) prenyl-9-BBN, Et_3N, THF, room temp., 5 h, 74%; (cc) Vilsmeier reagent, DMF, room temp., 2.5 h; then NaOH, 20 min, 93%; (dd) NaH, MOMCl, DMF, 0 °C to room temp., 25 h, 58%.

Therefore, we decided to install the prenyl group following a stepwise approach that we previously used in the synthesis of microphyllaquinone.[8] Accordingly, 5-bromoindole (**22**) was subjected to a Stille cross-coupling reaction with allyl(tributyl)stannane to give indole **23** in excellent yield. Subsequent cross-metathesis with 2-methyl-2-butene afforded the prenylated indole **24**. A reverse prenylation was carried out following a literature protocol to yield **25**.[12] Vilsmeier formylation then gave compound **26**, whose structure was confirmed by X-ray crystal structure analysis. Fi-

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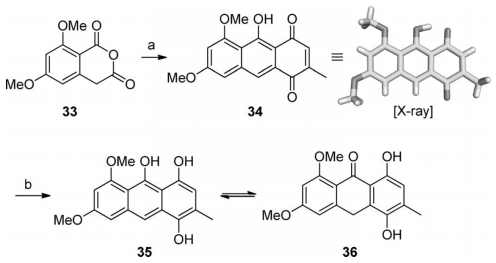
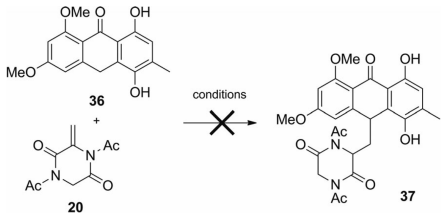
nally, **26** was protected to give MOM ether **27**. Analogously, formyl indole **32**, required for the synthesis of variecolortide B and C, was prepared in four steps starting from indole (**28**, see Scheme 3).

Our synthesis of the northern anthrone building block, compound **34**, closely followed the pioneering work of Caliskan[13] and is shown in Scheme 4. It started from known anhydride **33**,[14] which was treated with sodium hydride to generate the corresponding enolate that underwent a regiocontrolled cycloaddition reaction with 2-chloro-5-methylbenzoquinone. An aromatization of this intermediate, accompanied by the loss of CO_2 and HCl, provided hydroxyviocristin dimethyl ether (**34**) in good yield as a single regioisomer. The structure of the product was confirmed by X-ray crystal structure analysis. The required reduction of quinone **34** into the corresponding hydroquinone turned out to be rather difficult, since **34** was found to be insoluble in most solvents. Eventually, we found that the reduction of **34** in a mixture of chloroform, acetone, and water using sodium dithionite under sonication gave rise to a mixture of the desired anthrone **35** and its tautomer **36** (see Scheme 4). We found that **35** and **36** were extremely prone to reoxidize and give quinone **34**. As such, they could not be isolated and stored under ambient atmosphere, but instead had to be freshly prepared and used directly in the next step.



Scheme 4. Synthesis of **35**/**36** and attempted linkage. Reagents and conditions: (a) 2-chloro-5-methyl-benzoquinone, NaH, THF, 0 °C to room temp., 52%; (b) $Na_2S_2O_4$, $CHCl_3$/acetone/H_2O (1:1:2, degassed), sonication, 40 °C, 10 min.

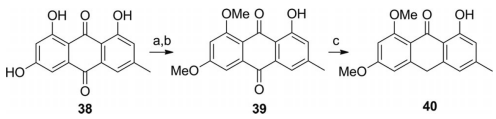
We next explored the Michael-type addition of anthrone **36** to diketopiperazine **20** (see Scheme 5). Although we had hoped that the deprotonation of **36** would give rise to a



Scheme 5. Attempted linkage of anthrone **36** and diketopiperazine **20**.

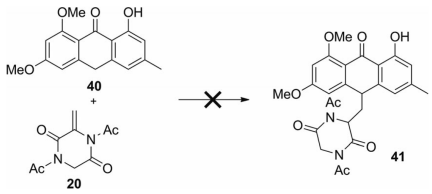
nucleophile that could undergo addition to the *exo*-methylene group of diketopiperazine **20**, we found that none of the conditions tested yielded any of the desired addition product **37**.

In addition to enabling the reoxidation of **36** to **34**, the phenolic hydroxy group in the 4-position could also interfere with the desired reaction through an unfavorable *peri* interaction. Therefore, we decided to remove it and try the critical linkage with the sterically less hindered emodin anthrone **40** (see Scheme 6). Our synthesis of this building block commenced with the triple methylation of the natural product emodin.[15] To allow for the planned oxidation of the phenol into the corresponding quinone, we had to selectively deprotect the methoxy group at C-8. This was achieved in good yield by treatment with BBr_3 at lower temperatures. Finally, the chemoselective reduction of anthraquinone **39** using tin(II) chloride yielded anthrone **40** (see Scheme 6).



Scheme 6. Synthesis of emodin anthrone **40**. Reagents and conditions: (a) K_2CO_3, Me_2SO_4, acetone, room temp., 30 min; then 90 °C, 16 h, 76%; (b) BBr_3, CH_2Cl_2, from –5 °C to room temp., 1 h, 73%; (c) $SnCl_2{\cdot}2H_2O$, HCl, AcOH, 65 °C, 1 h, 82%.

With the desired partially deoxygenated anthrone **40** in hand, we set out to screen for conditions that would effect a Michael addition to diketopiperazine **20** (see Scheme 7). Unfortunately, neither the use of bases, such as K_2CO_3, Et_3N, DBU (1,8-diazabicyclo[5.4.0]undec-7-ene), and *n*BuLi, nor the use of Lewis acids facilitated the required transformation. This led us to conclude that the *exo*-methylene diketopiperazines of type **20** are poor electrophiles, even with additional electron-withdrawing substituents on the nitrogen.



Scheme 7. Attempted monoalkylation of emodin anthrone **40** under nucleophilic conditions.

At that point, we became aware of the possibility of using radical chemistry, since anthrones are well known to form radicals in the presence of oxygen. We hypothesized that the central spirocycle of the variecolortides could arise from emodin anthrone **40** and *exo*-methylene diketopiperazine **20** by radical addition and subsequent oxidation reaction. To pursue such a biomimetic approach, we mixed both building blocks and stirred them under an oxygen atmo-

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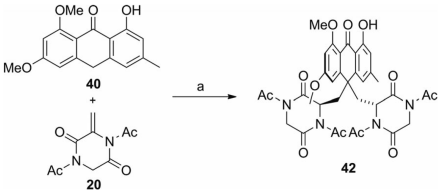
sphere (see Scheme 8). To our surprise, this led to a double addition reaction to give the doubly alkylated anthrone **42**, which proved to be crystalline. Its X-ray crystal structure is also shown in Figure 2. Remarkably, this unusual compound features a nonstereogenic chirotopic center. It is presumably formed through an initial hydrogen abstraction from **40**, followed by addition to **20**. This is followed by another hydrogen abstraction, another addition reaction, and a final abstraction. It is likely that stereoisomers of **42** are formed in the course of this process, but they could not be isolated.



Scheme 8. A surprising double-radical addition reaction. Reagents and conditions: (a) O_2, DMSO/H_2O (2:1), room temp., 20 h, 40%.

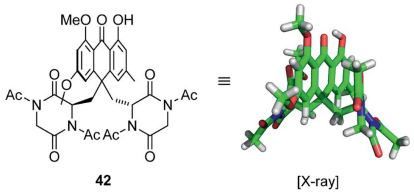
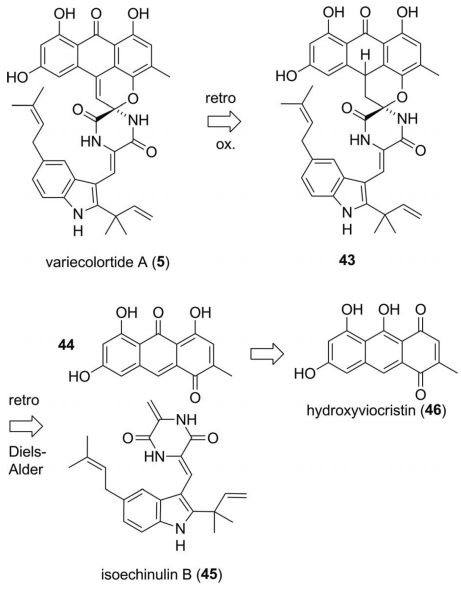


Figure 2. X-ray crystal structure of bis(diketopiperazine) **42**.

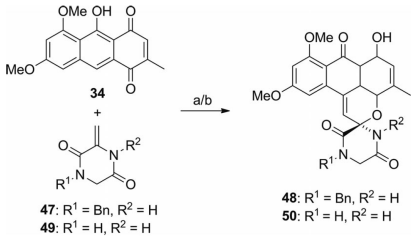
Subsequently, we tried to achieve a monoaddition reaction by varying several of the reaction parameters, such as stoichiometry, reaction time, the speed of the addition of the reagent, and so forth, but none of these changes led to the desired monoalkylated product **41**. Interestingly, if solvents other than DMSO (dimethyl sulfoxide) or other radical-generating conditions such as AIBN [azobis(isobutyronitrile)]/*n*Bu_3SnH were attempted, the dialkylated product **42** was not observed. Attempts to remove one of the diketopiperazines by using a base-induced elimination proved equally unsuccessful.

At this stage, we chose to abandon our original synthetic plan and adopt a different approach for the linkage of our building blocks. This eventually led us to consider one of our favorite reactions, that is, a hetero-Diels–Alder reaction, which had also played a prominent role in our total synthesis of rubioncolin B. The implementation of this reaction in the retrosynthesis of variecolortide A (**5**) is shown in Scheme 9. We hypothesized that the natural product could stem from the reduced anthrone precursor **43**, which could easily undergo an oxidative unsaturation in the presence of oxygen by virtue of its extremely labile C–H bond in the bis(benzylic) position. Compound **43**, a benzopyran, could then be retrosynthetically disassembled through a Diels–Alder disconnection to yield the *ortho*-quinone methide **44** and *exo*-methylene diketopiperazine **45**. The latter is a natural product known as isoechinulin B.[16] The reactive heterodiene **44**, in turn, would be formed by an intramolecular 1,5-hydrogen shift from an anthrone that is also a known natural product, hydroxyviocristin (**46**).



Scheme 9. Second-generation retrosynthetic analysis of variecolortide A (**5**).

To test our revised plan, we first heated a mixture of hydroxyviocristin dimethyl ether (**34**) and benzyl-protected *exo*-methylene diketopiperazine **47** in an aerated *ortho*-dichlorobenzene solution inside a pressure tube (see Scheme 10). Indeed, under these conditions, we isolated compound **48**, which displays the crucial pyrano-anthrone core of the variecolortides. Next, we tested the analogous reaction with the unprotected *exo*-methylene diketopiperazine **49**[7] and also obtained the corresponding cycloadduct **50** in good yield. The synthesis of **47**, an analogue of *exo*-



Scheme 10. Model system for the cycloaddition approach. Reagents and conditions: (a) *ortho*-dichlorobenzene, air, 210 °C, 1 h, 39%; (b) *ortho*-dichlorobenzene, air, 180 °C, 1.5 h, 73%.

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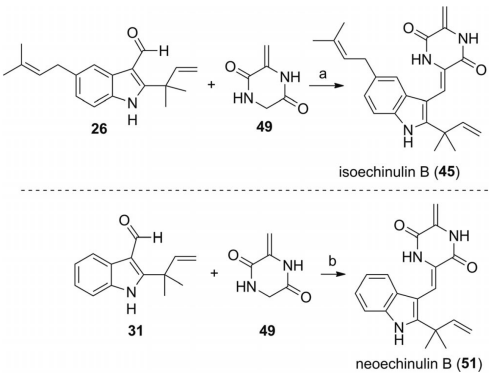
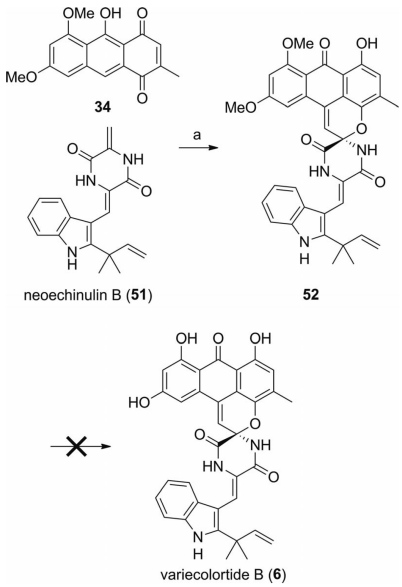
methylene diketopiperazine **20** is detailed in the Supporting Information.

Encouraged by these model studies, we proceeded to synthesize the three variecolortides, using isoechinulin B (**45**) or its desprenyl derivative neoechinulin B (**51**)[17] as dienophiles. The syntheses of these naturally occurring diketopiperazines using well-established routes are shown in Scheme 11.[7] We also obtained an X-ray crystal structure of isoechinulin B (**45**), albeit in poor quality (see Supporting Information).



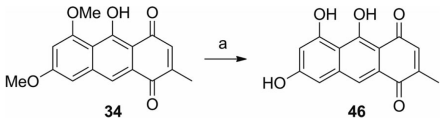
Scheme 11. Synthesis of isoechinulin B (**45**) and neoechinulin B (**51**). Reagents and conditions: (a) piperidine, 110 °C, 7 h, 39%; (b) piperidine, 110 °C, 7 h, 36%.



Scheme 12. Synthesis of dimethylvariecolortide B (**52**) and attempted demethylation. Reagents and conditions: (a) *ortho*-dichlorobenzene, air, 180 °C, 1.25 h, 34%.

When neoechinulin **51** was heated with readily available viocristin dimethyl ether **34** in a sealed tube in the presence of molecular oxygen, the corresponding dimethyl ether **52** of the natural product variecolortide B was obtained as depicted in Scheme 12. What remained to be done in order to finish the total synthesis was simply deprotection of the methyl groups. Considering the vast number of methods suitable for the deprotection of phenolic methyl ethers, we felt that this final step should be straightforward. However, under all of the conditions examined, we observed either decomposition or obtained a mixture of monodemethylated product and starting material.

Consequently, we decided to first carry out the deprotection of hydroxyviocristin dimethyl ether (**34**) and use the resulting resorcinol **46** in the key step of the synthesis. This could be achieved using a procedure published by Caliskan et al. (see Scheme 13).[13]



Scheme 13. Demethylation of hydroxyviocristin dimethyl ether (**34**). Reagents and conditions: (a) $AlCl_3$/NaCl, 180 °C, 2 min, 75%.

With the requisite northern building block **46** in hand, we were finally in a position to synthesize variecolortide A and B (see Scheme 14). As in the model systems, the natural products could be isolated in decent yields, considering the complexity of the reaction cascade. Whether the conditions used (air, *o*-dichlorobenzene, 180 °C) can be deemed "biomimetic", however, is questionable.

Variecolortide C (**7**) was the final member of the family to succumb to total synthesis. It differs from variecolortide B in that it bears a methoxy group at C-7. As we anticipated that a selective methylation of variecolortide B in the required position would be difficult, we decided to synthesize acetyl variecolortide C (**55**), which could subsequently be deprotected (see Scheme 15). To this end, we first prepared the known anhydride **53**.[13] Treatment with sodium hydride generated the corresponding enolate, which underwent a regiocontrolled cycloaddition reaction to 2-chloro-5-methyl-benzoquinone. An aromatization reaction accompanied by the loss of CO_2 and HCl provided acetyl viocristin **54** in 55% yield. Heating of **54** and neoechinulin B (**51**) in *ortho*-dichlorobenzene at 180 °C in the presence of oxygen afforded acetyl variecolortide C (**55**), which was subsequently converted into the desired natural product **7** by hydrolysis of the acetyl group. With this, we achieved a six-step synthesis of variecolortide C. Synthetic variecolortide C (**7**) exhibited spectroscopic data consistent with its structure and in accordance with those reported for the natural product.[6]

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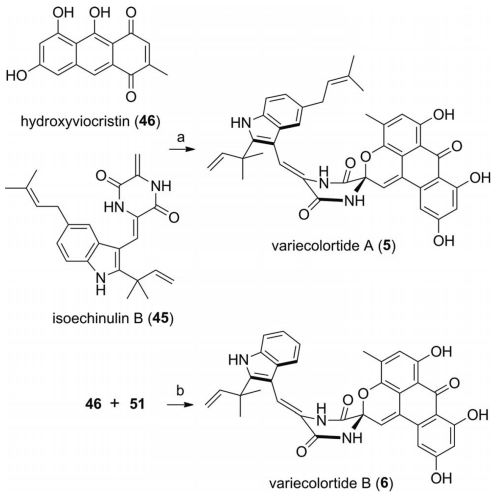
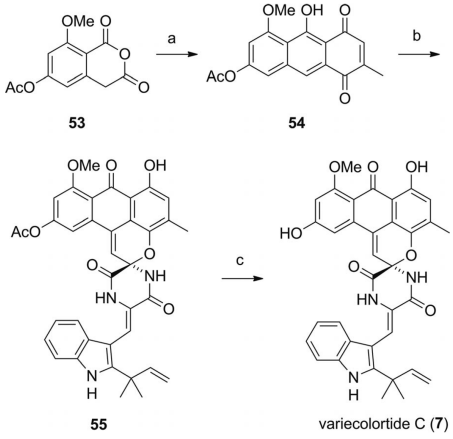


Scheme 14. Synthesis of variecolortide A (**5**) and B (**6**). Reagents and conditions: (a) *ortho*-dichlorobenzene, 180 °C, 0.5 h, 48%; (b) *ortho*-dichlorobenzene, 180 °C, 1 h, 32%.



Scheme 15. Synthesis of variecolortide C (**7**). Reagents and conditions: (a) 2-chloro-5-methyl-benzoquinone, NaH, THF, 0 °C to room temp., 55%; (b) neoechinulin B (**51**), *ortho*-dichlorobenzene, air, 180 °C, 1 h; (c) K_2CO_3, MeOH, room temp., 1 h, 28% over 2 steps.

Conclusions

In summary, we have presented a detailed account of our recent work on the variecolortides. Initial attempts to implement an oxa-6π-electrocyclization reaction proved unsuccessful, but led to important insights concerning the reactivities of *exo*-methylene diketopiperazines and anthrones. In addition, during these unsuccessful studies, several building blocks were assembled that could ultimately be used in our successful cycloaddition–oxidation approach that also yielded variecolortide C as a final member of the variecolortide family.

Experimental Section

General Methods: Unless stated otherwise, all of the reactions were performed in oven-dried or flame-dried glassware under a positive pressure of nitrogen. Commercial reagents and solvents were used as received with the following exceptions. Tetrahydrofuran was distilled from benzophenone and sodium immediately prior to use. Triethylamine, diisopropylamine, and diisopropylethylamine were distilled from calcium hydride immediately before use. The reactions were magnetically stirred and monitored by NMR spectroscopy or analytical thin-layer chromatography (TLC) using E. Merck 0.25 mm silica gel 60 F_{254} precoated glass plates. The TLC plates were visualized by exposure to ultraviolet light (UV, 254 nm) and exposure to either an aqueous solution of ceric ammonium-molybdate (CAM), an aqueous solution of potassium permanganate ($KMnO_4$), an acidic solution of vanillin, or a solution of ninhydrin in ethanol followed by heating with a heat gun. Flash column chromatography was performed as described by Still et al. employing silica gel (60 Å, 40–63 μm, Merck) and a forced flow of the eluent at 1.3–1.5 bar pressure.[18] The yields refer to chromatographically and spectroscopically (^{1}H and ^{13}C NMR) pure material.

Instrumentation: Proton nuclear magnetic resonance (^{1}H NMR) spectroscopic data were recorded with Varian VNMRS 300, VNMRS 400, INOVA 400, or VNMRS 600 spectrometers. Proton chemical shifts are expressed in parts per million (δ scale) and are calibrated by using the residual undeuterated solvent as an internal reference ($CDCl_3$, δ = 7.26 ppm; [D_6]DMSO, δ = 2.50 ppm). The data for the ^{1}H NMR spectra are reported as chemical shift (δ, ppm) [multiplicity, coupling constant (Hz), integration]. The multiplicities are reported by s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br. = broad, app = apparent, or combinations thereof. The carbon nuclear magnetic resonance (^{13}C NMR) spectroscopic data were recorded with Varian VNMRS 300, VNMRS 400, INOVA 400, or VNMRS 600 spectrometers. The carbon chemical shifts are expressed in parts per million (δ scale) and are referenced to the carbon resonances of the solvent ($CDCl_3$, δ = 77.0 ppm; [D_6]DMSO, δ = 39.5 ppm). The infrared (IR) spectra were recorded with a Perkin–Elmer Spectrum BX II (FTIR System). IR data are reported in frequency of absorption (cm^{-1}). The mass spectroscopy (MS) experiments were performed with a Thermo Finnigan MAT 95 (EI) or a Thermo Finnigan LTQ FT (ESI) instrument.

Bis(Boc)-diketopiperazine 16: To a white suspension of **15** (200 mg, 0.77 mmol. 1.0 equiv.) in dry DMF (2 mL) was added Boc_2O (356 mg, 1.63 mmol, 2.1 equiv.) and DMAP (199 mg, 1.63 mmol, 2.1 equiv.) at room temp. The resulting clear, yellow solution was stirred for 1.5 h, and then EtOAc (2 mL) and a saturated aqueous solution of $KHSO_4$ (4 mL) were added. The mixture was extracted with EtOAc (3 × 8 mL), and the combined organic layers were washed with a saturated aqueous solution of NaCl (8 mL), dried with sodium sulfate, filtered, and concentrated in vacuo. Purification of the crude product by flash column chromatography (silica

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gel, hexanes/EtOAc, 10:1) afforded the title compound **16** (244 mg, 0.53 mmol, 69%) as a white solid; m.p. 98–102 °C. R_f = 0.58 (hexanes/EtOAc, 10:1; $KMnO_4$). ^1H NMR (600 MHz, $CDCl_3$): δ = 4.74 (dd, J = 2.9, 1.8 Hz, 1 H), 4.50 (d, J = 17.9 Hz, 1 H), 4.31 (d, J = 17.9 Hz, 1 H), 4.21 (dd, J = 10.6, 1.8 Hz, 1 H), 4.02 (dd, J = 10.6, 2.9 Hz, 1 H), 1.53 (s, 9 H), 1.53 (s, 9 H), 0.85 (s, 9 H), 0.03 (s, 3 H), 0.03 (s, 3 H) ppm. ^{13}C NMR (150 MHz, $CDCl_3$): δ = 167.2, 165.3, 150.3, 149.9, 84.7, 84.7, 65.7, 62.4, 49.3, 27.9, 27.9, 25.8, 18.4, –5.7, –5.9 ppm. IR [diamond-ATR (attenuated total reflectance), neat]: $\tilde{\nu}_{max}$ = 2981, 2934, 2885, 2859, 1773, 1724, 1697, 1472, 1393, 1368, 1279, 1250, 1226, 1144, 1115, 1086, 1054, 1035, 995, 940, 878, 834, 811, 776, 709 cm^{-1}. HRMS (EI): calcd. for $C_{21}H_{38}N_2O_7Si$ $[M]^{+\cdot}$ 458.2448; found 458.2450.

(*S*)-3-{[(*tert*-Butyldimethylsilyl)oxy]methyl}-1,4-bis(4-methoxybenzyl)piperazine-2,5-dione (S1): To a suspension of sodium hydride (60% dispersion in mineral oil, 173 mg, 4.33 mmol, 2.05 equiv.) in dry DMF (40 mL) was added piperazinedione **15** (546 mg, 2.11 mmol, 1.0 equiv.) at 0 °C. After 10 min, 4-methoxybenzyl chloride (661 mg, 4.22 mmol, 2.0 equiv.) was added dropwise, and the reaction mixture was stirred for another 2 h at 0 °C. The resulting light yellow solution was warmed to room temp., and the stirring was continued for another 12 h. Water (150 mL) was added, and the white mixture was extracted with EtOAc (3 × 100 mL). The combined organic layers were dried with sodium sulfate, filtered, and concentrated in vacuo. Purification of the residue by flash column chromatography (silica gel, hexanes/EtOAc, 5:1) afforded the title compound **S1** (561 mg, 1.12 mmol, 53%) as a white solid; m.p. 112 °C. R_f = 0.52 (hexanes/EtOAc, 1:1; UV/CAM). ^1H NMR (600 MHz, $CDCl_3$): δ = 7.18 (dd, J = 10.9, 8.6 Hz, 4 H), 6.85–6.83 (m, 4 H), 5.15 (d, J = 14.8 Hz, 1 H), 4.78 (d, J = 14.4 Hz, 1 H), 4.24 (d, J = 14.4 Hz, 1 H), 4.05–4.04 (m, 1 H), 4.02 (s, 1 H), 3.96 (d, J = 14.8 Hz, 1 H), 3.88 (s, 1 H), 3.84 (dd, J = 10.4, 2.7 Hz, 1 H), 3.79 (s, 3 H), 3.79 (s, 3 H), 3.74 (d, J = 16.9 Hz, 1 H), 0.78 (s, 9 H), –0.05 (s, 3 H), –0.06 (s, 3 H) ppm. ^{13}C NMR (150 MHz, $CDCl_3$): δ = 165.9, 165.4, 159.4, 159.4, 129.8, 129.7, 127.5, 127.2, 114.3, 114.2, 62.8, 61.5, 55.3, 55.3, 49.6, 49.0, 46.4, 25.7, 18.2, –5.7, –5.8 ppm. IR (diamond-ATR, neat): $\tilde{\nu}_{max}$ = 2930, 2853, 2360, 2341, 1651, 1612, 1584, 1512, 1467, 1434, 1354, 1322, 1306, 1247, 1210, 1179, 1164, 1123, 1110, 1087, 1059, 1033, 1011, 952, 912, 868, 842, 826, 810, 781, 769, 728, 702, 668 cm^{-1}. HRMS (EI): calcd. for $C_{27}H_{38}N_2O_5Si$ $[M]^{+\cdot}$ 498.2550; found 498.2537.

Alcohol 18: To a solution of **S1** (245 mg, 0.49 mmol, 1.0 equiv.) in dry MeOH (20 mL) was added NH_4F (0.5 M solution in MeOH, 14.7 mL, 7.35 mmol, 15 equiv.), and the solution was heated to 40 °C and stirred at this temperature for 5 d. After cooling to room temp., the reaction mixture was concentrated to half of its volume, and an equal amount of saturated aqueous $NaHCO_3$ was added. The mixture was extracted with EtOAc (3 × 25 mL), and the combined organic extracts were dried with sodium sulfate and filtered. The solvent was evaporated under reduced pressure. Purification of the residue by flash column chromatography (silica gel, $CHCl_3$/acetone, 2:1) afforded the title compound **18** (188 mg, 0.49 mmol, 99%) as a white solid. R_f = 0.37 ($CHCl_3$/acetone, 2:1; UV/CAM). ^1H NMR (600 MHz, $CDCl_3$): δ = 7.17–7.15 (m, 4 H), 6.85 (d, J = 8.3 Hz, 4 H), 5.12 (d, J = 14.8 Hz, 1 H), 4.61 (d, J = 14.6 Hz, 1 H), 4.41 (d, J = 14.6 Hz, 1 H), 4.06 (d, J = 6.5 Hz, 1 H), 4.03 (d, J = 4.1 Hz, 1 H), 4.00 (dd, J = 10.8, 1.4 Hz, 1 H), 3.89–3.85 (m, 2 H), 3.78 (s, 3 H), 3.78 (s, 3 H), 3.73 (d, J = 17.2 Hz, 1 H), 2.83 (br. s, 1 H) ppm. ^{13}C NMR (150 MHz, $CDCl_3$): δ = 165.9, 165.4, 159.4, 159.3, 129.6, 129.5, 127.3, 127.0, 114.4, 114.2, 62.1, 61.3, 55.3, 55.2, 49.2, 48.8, 46.5 ppm. IR (diamond-ATR, neat): $\tilde{\nu}_{max}$ = 3403, 2924, 2837, 2360, 2342, 1901, 1650, 1611, 1584, 1511, 1468, 1440, 1417, 1362, 1321, 1264, 1247, 1177, 1165, 1102, 1073, 1026, 976, 960, 938, 892, 852, 823, 813, 766, 703, 668 cm^{-1}. HRMS (EI): calcd. for $C_{21}H_{24}N_2O_5$ $[M]^{+\cdot}$ 384.1685; found 384.1676.

MOM-Protected Indole 27: A solution of indole **26** (427 mg, 1.52 mmol, 1.0 equiv.) in dry DMF (5 mL) was added to a suspension of sodium hydride (60% dispersion in mineral oil, 608 mg, 15.2 mmol, 10 equiv.) in dry DMF (5 mL) at 0 °C. After stirring the suspension at 0 °C for 30 min, chloromethyl methyl ether (1.15 mL, 15.2 mmol, 10 equiv.) was added dropwise. The reaction mixture was warmed to room temp., stirred for another 20 h, and then quenched by the addition of a saturated aqueous solution of NH_4Cl (20 mL). The orange suspension was extracted with EtOAc (3 × 50 mL), and the combined organic layers were dried with sodium sulfate, filtered, and concentrated under reduced pressure. Purification of the crude orange oil by flash column chromatography (silica gel, hexanes/EtOAc, 5:1) afforded the title compound **27** (426 mg, 1.31 mmol, 86%) as a yellow oil. R_f = 0.74 (hexanes/EtOAc, 2:1; UV/CAM). ^1H NMR (600 MHz, $CDCl_3$): δ = 10.67 (s, 1 H), 8.33 (dd, J = 1.5, 0.6 Hz, 1 H), 7.30 (d, J = 8.4 Hz, 1 H), 7.13 (dd, J = 8.4, 1.7 Hz, 1 H), 6.28 (dd, J = 17.5, 10.6 Hz, 1 H), 5.52 (s, 2 H), 5.39–5.36 (m, 1 H), 5.15 (d, J = 10.6 Hz, 1 H), 5.08 (d, J = 17.5 Hz, 1 H), 3.46 (d, J = 7.3 Hz, 2 H), 3.36 (s, 3 H), 1.76 (d, J = 0.9 Hz, 3 H), 1.75 (s, 6 H), 1.74 (d, J = 1.2 Hz, 3 H) ppm. ^{13}C NMR (150 MHz, $CDCl_3$): δ = 188.6, 153.7, 146.9, 137.2, 136.2, 131.8, 126.4, 124.7, 124.2, 121.9, 117.4, 112.5, 109.7, 75.6, 55.8, 42.0, 34.5, 30.8, 25.8, 17.9 ppm. IR (diamond-ATR, neat): $\tilde{\nu}_{max}$ = 2973, 2927, 1644, 1478, 1456, 1374, 1347, 1316, 1223, 1196, 1178, 1148, 1084, 1041, 1024, 966, 914, 874, 844, 799, 726, 679 cm^{-1}. HRMS (EI): calcd. for $C_{21}H_{27}NO_2$ $[M]^{+\cdot}$ 325.2042; found 325.2032.

MOM-Protected Indole 32: Sodium hydride (60% dispersion in mineral oil, 936 mg, 23.4 mmol, 10 equiv.) was added to a solution of indole **31** (500 mg, 2.34 mmol, 1.0 equiv.) in dry DMF (12 mL) at 0 °C, and the resulting suspension was stirred at this temperature for 30 min. Chloromethyl methyl ether (1.78 mL, 23.4 mmol, 10 equiv.) was added dropwise, and the reaction mixture was warmed to room temp. and then stirred for 25 h. A saturated aqueous solution of NH_4Cl (50 mL) was added, and the mixture was extracted with EtOAc (4 × 35 mL). The combined organic layers were washed with a saturated aqueous solution of NaCl (50 mL), dried with sodium sulfate, filtered, and concentrated in vacuo. Purification of the crude product by flash column chromatography (silica gel, hexanes/EtOAc, 10:1) yielded the title compound **32** (349 mg, 1.36 mmol, 58%) as a yellow oil. R_f = 0.57 (hexanes/EtOAc, 5:1; UV/CAM). ^1H NMR (300 MHz, $CDCl_3$): δ = 10.70 (s, 1 H), 8.53–8.48 (m, 1 H), 7.42–7.36 (m, 1 H), 7.34–7.28 (m, 1 H), 6.30 (dd, J = 17.5, 10.6 Hz, 1 H), 5.55 (s, 2 H), 5.17 (d, J = 10.6 Hz, 1 H), 5.09 (d, J = 17.5 Hz, 1 H), 3.38 (s, 3 H), 1.77 (s, 6 H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 188.6, 153.5, 146.9, 146.8, 137.6, 126.3, 124.0, 123.3, 122.7, 117.5, 112.6, 109.8, 75.5, 55.8, 42.0, 30.8 ppm. IR (diamond-ATR, neat): $\tilde{\nu}_{max}$ = 2974, 2934, 2900, 1640, 1584, 1511, 1501, 1467, 1406, 1375, 1349, 1221, 1204, 1191, 1125, 1082, 1064, 1040, 990, 964, 921, 818, 798, 749, 740, 718, 688, 668 cm^{-1}. HRMS (ESI): calcd. for $C_{16}H_{19}NO_2Na$ $[M + Na]^+$ 280.1308; found 280.1309.

1,3-Dimethoxy-8-hydroxy-6-methyl-9,10-anthraquinone (39): To a solution of 1,3,8-trimethoxy-6-methylanthracene-9,10-dione[15] (288 mg, 0.92 mmol, 1.0 equiv.) in CH_2Cl_2 (13 mL) was added BBr_3 (1.0 M in CH_2Cl_2, 2.86 mL, 2.86 mmol, 3.1 equiv.) dropwise at –5 °C. The dark red reaction mixture was warmed to room temp. and stirred for 1 h. The mixture was then poured into ice/H_2O, and

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the resulting mixture was stirred for an additional 30 min. The layers were separated, and the aqueous layer was extracted with CH_2Cl_2 (3×15 mL). The combined organic layers were dried with sodium sulfate, filtered, and concentrated under reduced pressure. The resulting red-brown solid was then purified by flash column chromatography (silica gel, $CHCl_3$/acetone, 100:1 → 25:1) to afford **39** (200 mg, 0.67 mmol, 73%) as a bright orange solid; m.p. 202–205 °C. R_f = 0.64 ($CHCl_3$/acetone, 20:1; UV/CAM). ^{1}H NMR (600 MHz, $CDCl_3$): δ = 7.55 (dd, J = 1.5, 0.4 Hz, 1 H), 7.44 (d, J = 2.5 Hz, 1 H), 7.06 (dd, J = 1.6, 0.8 Hz, 1 H), 6.77 (d, J = 2.5 Hz, 1 H), 4.02 (s, 3 H), 3.98 (s, 3 H), 2.42 (s, 3 H) ppm. ^{13}C NMR (150 MHz, $CDCl_3$): δ = 187.4, 182.9, 165.2, 162.9, 162.6, 146.9, 137.6, 132.3, 124.8, 120.0, 115.2, 114.7, 104.7, 103.9, 56.6, 56.0, 22.0 ppm. IR (diamond-ATR, neat): $\tilde{\nu}_{max}$ = 2946, 2845, 1669, 1626, 1592, 1553, 1493, 1362, 1322, 1263, 1218 cm^{-1}. HRMS (ESI): calcd. for $C_{17}H_{15}O_5$ $[M + H]^+$ 299.0914; found 299.0914.

8-Hydroxy-1,3-dimethoxy-6-methyl-10*H*-anthracen-9-one (40): A solution of **39** (573 mg, 1.92 mmol, 1.0 equiv.) in glacial acetic acid (75 mL) was heated to 65 °C. After the addition of a solution of $SnCl{\cdot}2H_2O$ (3.47 g, 15.4 mmol, 8 equiv.) in concentrated aqueous HCl (15 mL), the resulting mixture was stirred at 65 °C. After 1 h, the reaction mixture was poured into ice/H_2O (100 mL), and the resulting mixture was stirred for an additional hour. The mixture was then extracted with $CHCl_3$ (2× 70 mL), and the combined organic layers were washed with water (100 mL), dried with sodium sulfate, and concentrated in vacuo. Purification of the residue by flash column chromatography (silica gel, $CHCl_3$/acetone, 100:1 → 5:1) provided **40** (450 mg, 1.58 mmol, 82%) as a brown solid; m.p. 176–180 °C. R_f = 0.54 ($CHCl_3$/acetone, 20:1; UV/CAM). ^{1}H NMR (600 MHz, $CDCl_3$): δ = 13.32 (s, 1 H), 6.66–6.61 (m, 2 H), 6.47–6.44 (m, 2 H), 4.23 (s, 2 H), 3.97 (s, 3 H), 3.89 (s, 3 H), 2.33 (s, 3 H) ppm. ^{13}C NMR (150 MHz, $CDCl_3$): δ = 188.7, 164.2, 163.3, 162.8, 146.0, 145.7, 139.8, 118.5, 115.7, 115.3, 114.6, 104.2, 97.7, 56.2, 55.5, 33.9, 21.9 ppm. IR (diamond-ATR, neat): $\tilde{\nu}_{max}$ = 3018, 2951, 1630, 1599, 1504, 1334, 1257, 1224, 1094, 825 cm^{-1}. HRMS (EI): calcd. for $C_{17}H_{16}O_4$ $[M]^{+\cdot}$ 284.1043; found 284.1043.

Radical Adduct 42: To a solution of **40** (10 mg, 35 µmol, 1.0 equiv.) in a mixture of DMSO (1.0 mL) and water (0.5 mL) was added **20** (8.1 mg, 38.5 µmol, 1.1 equiv.). The inner atmosphere of the flask was exchanged with oxygen, and then the reaction mixture was allowed to stir under an O_2 atmosphere (double-layer balloon) at room temp. After 20 h, the reaction mixture was concentrated in vacuo, and the residue was purified by flash column chromatography (silica gel, $CHCl_3$/acetone, 50:1 → 1:1) to afford **42** (9.8 mg, 1.39 µmol, 40%) as a 1:1 mixture of diastereoisomers. R_f = 0.36 ($CHCl_3$/acetone, 3:1; UV/CAM). ^{1}H NMR (600 MHz, $CDCl_3$): δ = 13.98 (s, 1 H), 13.89 (s, 1 H), 6.77 (d, J = 2.2 Hz, 1 H), 6.75–6.74 (m, 2 H), 6.68–6.66 (m, 4 H), 6.59 (d, J = 2.2 Hz, 1 H), 4.90–4.84 (m, 3 H), 4.82 (s, 1 H), 4.72 (dd, J = 8.8, 6.6 Hz, 1 H), 4.69 (dd, J = 10.6, 5.9 Hz, 2 H), 4.61 (dd, J = 11.2, 5.6 Hz, 1 H), 4.07 (s, 3 H), 4.05 (s, 3 H), 4.04 (s, 3 H), 3.98 (s, 3 H), 3.82 (dd, J = 18.7, 5.8 Hz, 2 H), 3.79 (d, J = 18.7 Hz, 2 H), 2.67–2.60 (m, 4 H), 2.53–2.48 (m, 4 H), 2.41 (s, 3 H), 2.37 (s, 6 H), 2.36 (s, 3 H), 2.27 (s, 3 H), 2.27 (s, 3 H), 2.19 (s, 3 H), 2.10 (s, 6 H), 2.08 (s, 3 H) ppm. ^{13}C NMR (150 MHz, $CDCl_3$): δ = 187.5, 187.5, 170.8, 170.7, 170.7, 170.5, 170.1, 165.8, 165.7, 165.7, 165.4, 165.3, 165.0, 164.6, 164.4, 163.7, 163.7, 163.4, 163.3, 146.5, 145.9, 145.6, 145.1, 141.0, 140.2, 118.0, 117.9, 117.7, 116.6, 116.3, 115.4, 115.4, 114.2, 102.3, 102.0, 99.7, 98.7, 56.5, 56.4, 55.9, 55.8, 55.0, 54.8, 54.3, 48.3, 48.0, 47.5, 46.7, 46.6, 46.6, 42.3, 42.3, 26.6, 26.6, 26.5, 26.5, 26.5, 26.3, 22.1, 22.0 ppm. IR (diamond-ATR, neat): $\tilde{\nu}_{max}$ = 3009, 2938, 2846, 1706, 1631, 1597, 1568, 1498, 1453, 1416, 1366, 1325, 1310, 1253, 1201, 1165, 1136, 1039, 983, 949, 896, 851 cm^{-1}. HRMS (ESI): calcd. for $C_{35}H_{37}N_4O_{12}$ $[M + H]^+$ 705.2402; found 704.2406.

1-Benzyl-3-methylenepiperazine-2,5-dione (47): To a solution of (*S*)-1-benzyl-3-(hydroxymethyl)piperazine-2,5-dione (400 mg, 1.7 mmol, 1.0 equiv.) in dry pyridine (17 mL) was added methanesulfonyl chloride (224 µL, 2.9 mmol, 1.7 equiv.) dropwise at 0 °C. The resulting yellow reaction mixture was stirred at this temperature for 2 h, and then MeOH (5 mL) was added. The solution was warmed to room temp., and the solvent was removed in vacuo. To the residue was added water (10 mL), and the aqueous layer was extracted with $CHCl_3$ (3× 10 mL). The combined organic layers were dried with sodium sulfate, filtered, and concentrated under reduced pressure to give the crude (*S*)-(4-benzyl-3,6-dioxopiperazin-2-yl)methyl methanesulfonate, which was directly used in the next step without further purification. The crude mesylate was dissolved in dry CH_2Cl_2 (25 mL), and triethylamine (547 µL) was added dropwise at room temp. The solution was stirred at room temp. for 20 h, and then a saturated aqueous solution of NH_4Cl (25 mL) was added. The organic layer was separated, dried with sodium sulfate, filtered, and concentrated in vacuo. Purification of the residue by flash column chromatography (silica gel, hexanes/EtOAc, 1:2) provided **47** (270 mg, 1.25 mmol, 73% over 2 steps) as a white solid; m.p. 163 °C dec. R_f = 0.33 (hexanes/EtOAc, 1:3; UV/CAM). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 9.19 (br. s, 1 H), 7.39–7.27 (m, 5 H), 5.70 (s, 1 H), 4.92 (d, J = 0.6 Hz, 1 H), 4.66 (s, 2 H), 3.99 (s, 2 H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 163.4, 157.3, 134.8, 132.8, 129.0, 128.6, 128.3, 103.4, 50.0, 49.5 ppm. IR (diamond-ATR, neat): $\tilde{\nu}_{max}$ = 3183, 3063, 3028, 2923, 1675, 1622, 1494, 1472, 1454, 1417, 1328, 1220, 1176, 1081, 1026, 978, 942, 855, 829, 800, 780, 756, 696, 654 cm^{-1}. HRMS (EI): calcd. for $C_{12}H_{12}N_2O_2$ $[M]^{+\cdot}$ 216.0899; found 216.0891.

Diels–Alder Adduct 48: No precautions were taken to exclude oxygen from the reaction vessel. To a mixture of 1,4-anthraquinone **34** (15 mg, 50.3 µmol, 1.0 equiv.) and diketopiperazine **47** (11 mg, 50.9 µmol, 1.01 equiv.) in a pressure tube was added *ortho*-dichlorobenzene (1.5 mL), and the dark purple reaction mixture was heated to 210 °C. After 1 h, the mixture was cooled to room temp. and directly subjected to flash column chromatography (silica gel, eluting first with hexanes to remove *o*-dichlorobenzene and then $CHCl_3$/acetone, 10:1 → 5:1 → 1:1) to afford the title compound **48** (10 mg, 19.5 µmol, 39%) as an orange solid. R_f = 0.50 ($CHCl_3$/MeOH, 10:1; UV/CAM). ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 13.02 (s, 1 H), 9.68 (s, 1 H), 7.42–7.38 (m, 3 H), 7.35–7.31 (m, 3 H), 7.25 (s, 1 H), 6.87 (d, J = 0.7 Hz, 1 H), 6.81 (d, J = 2.2 Hz, 1 H), 4.82 (d, J = 15.0 Hz, 1 H), 4.55 (d, J = 15.0 Hz, 1 H), 4.29 (d, J = 17.8 Hz, 1 H), 3.99 (s, 3 H), 3.96 (d, J = 17.8 Hz, 1 H), 3.92 (s, 3 H), 2.18 (d, J = 0.7 Hz, 3 H) ppm. ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ = 186.4, 166.5, 164.5, 163.3, 162.6, 156.2, 138.0, 137.6, 135.8, 134.2, 128.7, 127.4, 126.4, 120.5, 119.1, 116.6, 112.4, 110.4, 100.5, 100.3, 83.8, 56.2, 56.1, 49.5, 48.8, 15.5 ppm. IR (diamond-ATR, neat): $\tilde{\nu}_{max}$ = 2922, 2852, 1672, 1599, 1490, 1453, 1376, 1329, 1246, 1226, 1161, 1043, 1027, 979, 950, 926, 869, 822, 800, 732, 699 cm^{-1}. HRMS (EI): calcd. for $C_{29}H_{24}N_2O_7$ $[M]^{+\cdot}$ 512.1584; found 512.1575.

Dimethyl Variecolortide B (52): No precautions were taken to exclude oxygen from the reaction vessel. A pressure tube was charged with hydroxyviocristin dimethyl ether (**34**, 12.7 mg, 42.4 µmol, 1.0 equiv.), neoechinulin B (**51**, 15 mg, 46.7 µmol, 1.1 equiv.), and *ortho*-dichlorobenzene (1.5 mL). The pressure tube was sealed, and the reaction mixture was heated at 180 °C for 1.25 h and then subsequently cooled to room temp. The crude product was subjected directly to flash column chromatography (silica gel, eluting first

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with hexanes to remove *o*-dichlorobenzene and then $CHCl_3$/acetone, 20:1 → 10:1 → 1:1) to afford the title compound **52** (8.8 mg, 14.2 µmol, 34%) as an orange solid. R_f = 0.52 ($CHCl_3$/acetone, 5:1; UV/CAM). ^{1}H NMR (600 MHz, [D$_6$]DMSO): δ = 13.04 (s, 1 H), 11.14 (s, 1 H), 10.00 (s, 1 H), 9.56 (s, 1 H), 7.46–7.45 (m, 1 H), 7.45–7.44 (m, 1 H), 7.38 (d, *J* = 2.3 Hz, 1 H), 7.24 (s, 1 H), 7.17 (s, 1 H), 7.10 (ddd, *J* = 8.2, 7.1, 1.2 Hz, 1 H), 7.05 (ddd, *J* = 7.9, 7.1, 1.0 Hz, 1 H), 6.89 (d, *J* = 0.8 Hz, 1 H), 6.82 (d, *J* = 2.2 Hz, 1 H), 6.11 (dd, *J* = 17.6, 10.3 Hz, 1 H), 5.09 (dd, *J* = 17.6, 1.2 Hz, 1 H), 5.09 (dd, *J* = 10.3, 1.2 Hz, 1 H), 3.99 (s, 3 H), 3.93 (s, 3 H), 2.25 (d, *J* = 0.7 Hz, 3 H), 1.53 (s, 3 H), 1.49 (s, 3 H) ppm. ^{13}C NMR (150 MHz, [D$_6$]DMSO): δ = 186.5, 164.5, 163.3, 161.4, 161.4, 156.2, 145.1, 144.9, 137.9, 137.8, 135.2, 134.3, 126.6, 126.3, 123.7, 120.8, 120.0, 119.4, 119.4, 119.1, 116.9, 114.5, 112.4, 111.8, 111.5, 110.5, 103.9, 100.5, 100.3, 84.0, 56.2, 56.0, 40.4, 27.9, 27.4, 15.7 ppm. HRMS (ESI): calcd. for $C_{36}H_{33}N_3O_7$ [M – H]$^-$ 616.2089; found 616.2073.

Acetyl Viocristin 54: To a suspension of sodium hydride (60% dispersion in mineral oil, 22 mg, 0.55 mmol, 2.4 equiv.) in dry THF (5 mL) was added anhydride **53**[13] (57 mg, 228 µmol, 1.0 equiv.) at 0 °C, and the resulting suspension was stirred at this temperature for 20 min. Then, a solution of 2-chloro-5-methyl-1,4-benzoquinone (42.8 mg, 273 µmol, 1.2 equiv.) in dry THF (5 mL) was added at 0 °C. The red suspension was warmed to room temp., and a saturated aqueous solution of NH_4Cl (10 mL) was added. The reaction mixture was extracted with $CHCl_3$ (3×15 mL), and the combined organic layers were dried with sodium sulfate, filtered, and concentrated in vacuo. The residue was purified by flash column chromatography (silica gel, $CHCl_3$/acetone, 10:1) to yield **54** (41.2 mg, 126 µmol, 55%) as a purple solid; m.p. > 215 °C dec. R_f = 0.33 ($CHCl_3$/acetone, 80:1; UV/CAM). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 14.86 (s, 1 H), 7.91 (s, 1 H), 7.26 (s, 1 H), 6.87 (q, *J* = 1.5 Hz, 1 H), 6.79 (d, *J* = 2.1 Hz, 1 H), 4.03 (s, 3 H), 2.37 (s, 3 H), 2.19 (d, *J* = 1.5 Hz, 3 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 188.5, 184.4, 168.7, 164.8, 161.2, 153.0, 149.8, 139.1, 137.2, 128.5, 121.5, 116.2, 114.3, 109.0, 104.7, 56.5, 21.2, 16.6 ppm. IR (diamond-ATR, neat): $\tilde{\nu}_{max}$ = 3093, 3009, 2975, 2945, 2920, 2848, 2362, 2341, 1760, 1667, 1640, 1589, 1446, 1427, 1394, 1341, 1276, 1242, 1190, 1146, 1121, 1025, 1001, 975, 912, 890, 873, 824, 811, 782, 749, 702, 667, 628 cm^{-1}. HRMS (ESI): calcd. for $C_{18}H_{15}O_6$ [M + H]$^+$ 327.0869; found 327.0864.

Variecolortide C (7): Note: no precautions were taken to exclude oxygen from the reaction vessel. To a mixture of acetyl viocristin **54** (23.7 mg, 72.6 µmol, 1.1 equiv.) and neoechinulin B (**51**, 21.2 mg, 65.9 µmol, 1.0 equiv.) in a pressure tube was added *o*-dichlorobenzene (1.5 mL), and the dark purple reaction mixture was heated to 180 °C (in a preheated oil bath). After 1 h, the reaction mixture was cooled to room temp. and then directly subjected to flash column chromatography (silica gel, eluting first with hexanes to remove *o*-dichlorobenzene and then $CHCl_3$/acetone, 40:1 → 20:1 → 10:1→ 1:1) to afford acetyl variecolortide C (**55**), which was used directly in the next step without further purification. To an orange solution of **55** in MeOH (3 mL) was added potassium carbonate (8 mg) at room temp. The resulting brown reaction mixture was stirred for 1 h, and then saturated aqueous NH_4Cl (1.5 mL) and water (1.5 mL) were added. The mixture was extracted with $CHCl_3$ (3×15 mL), and the combined organic extracts were dried with sodium sulfate, filtered, and concentrated under reduced pressure. Purification of the residue by flash column chromatography (silica gel, $CHCl_3$/acetone, 10:1 → $CHCl_3$/MeOH, 10:1) afforded variecolortide C (**7**, 11 mg, 18.2 µmol, 28% over 2 steps) as an orange solid. R_f = 0.39 ($CHCl_3$/MeOH, 5:1; UV/CAM). ^{1}H NMR (600 MHz, [D$_6$]DMSO): δ = 13.12 (s, 1 H), 11.14 (s, 1 H), 10.89 (br. s, 1 H), 9.98 (s, 1 H), 9.55 (s, 1 H), 7.47 (d, *J* = 8.0 Hz, 1 H), 7.44 (d, *J* = 8.0 Hz, 1 H), 7.17 (d, *J* = 2.9 Hz, 1 H), 7.17 (s, 1 H), 7.11–7.09 (m, 1 H), 7.06–7.03 (m, 1 H), 6.87 (s, 1 H), 6.87 (s, 1 H), 6.68 (d, *J* = 2.0 Hz, 1 H), 6.11 (dd, *J* = 17.6, 10.3 Hz, 1 H), 5.09 (dd, *J* = 10.4, 1.1 Hz, 1 H), 5.09 (dd, *J* = 17.6, 1.1 Hz, 1 H), 3.88 (s, 3 H), 2.24 (s, 3 H), 1.53 (s, 3 H), 1.49 (s, 3 H) ppm. ^{13}C NMR (150 MHz, [D$_6$]DMSO): δ = 186.3, 163.7, 163.6, 161.6, 161.5, 156.2, 145.1, 144.9, 137.8, 137.6, 135.1, 134.1, 126.8, 126.3, 123.8, 120.8, 119.4, 119.4, 119.1, 118.9, 117.0, 114.5, 111.8, 111.5, 111.1, 110.4, 104.0, 102.4, 100.9, 83.9, 55.9, 40.0, 27.9, 27.3, 15.7 ppm. IR (diamond-ATR, neat): $\tilde{\nu}_{max}$ = 3220, 2967, 2925, 2360, 2342, 1685, 1615, 1578, 1456, 1365, 1333, 1239, 1222, 1207, 1170, 1121, 1049, 1022, 1001, 915, 899, 882, 820, 748 cm^{-1}. HRMS (ESI): calcd. for $C_{35}H_{30}N_3O_7$ [M + H]$^+$ 604.2078; found 604.2089.

CCDC-883797 (for **16**), -883798 (for **20**), -883796 (for **26**), -883856 (for **34**), -883799 (for **42**) contain the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

Supporting Information (see footnote on the first page of this article): Copies of the ^{1}H and ^{13}C NMR spectra for all of the key intermediates and final products as well as the X-ray crystal structures.

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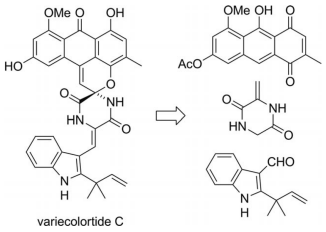
Total Synthesis

Variations on a theme by *Aspergillus variecolor*: The concise total syntheses of the variecolortides, in particular of variecolortide C, are presented.



Keywords: Total synthesis / Natural products / Cycloaddition / Biomimetic synthesis / Domino reactions / Cascade reactions

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SUPPORTING INFORMATION

DOI: 10.1002/ejoc.201200712
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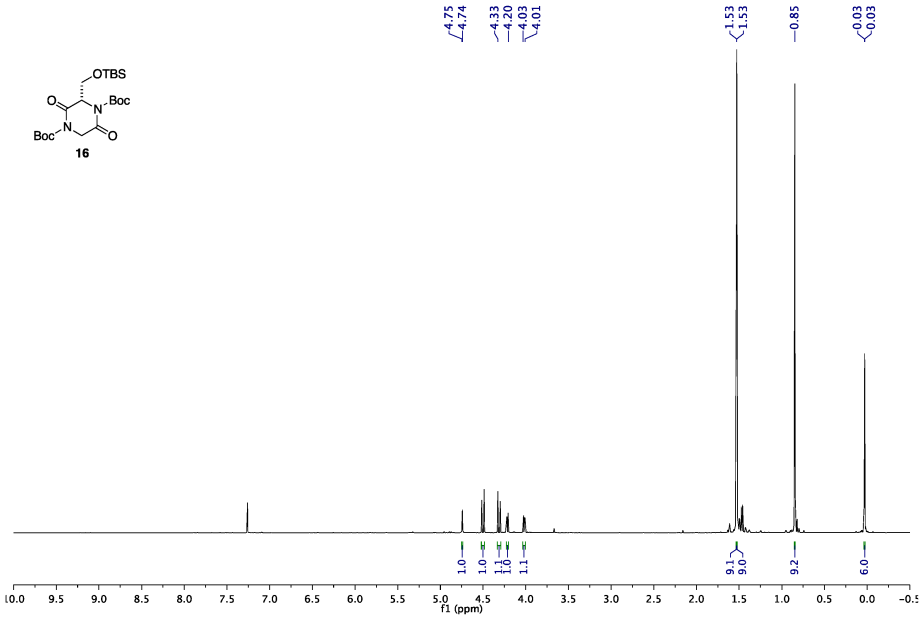
Author(s): Christian A. Kuttruff, Peter Mayer, Dirk Trauner*

NMR Spectra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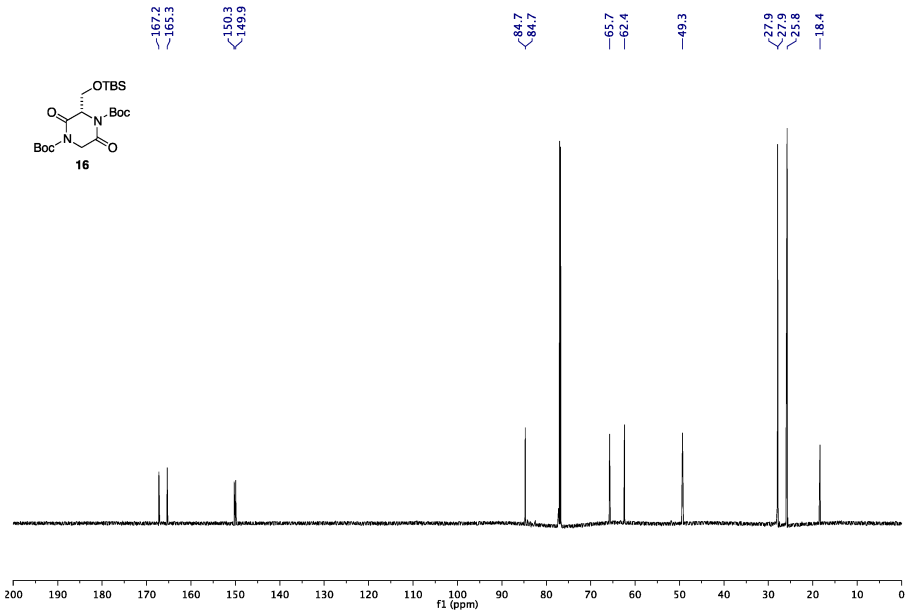
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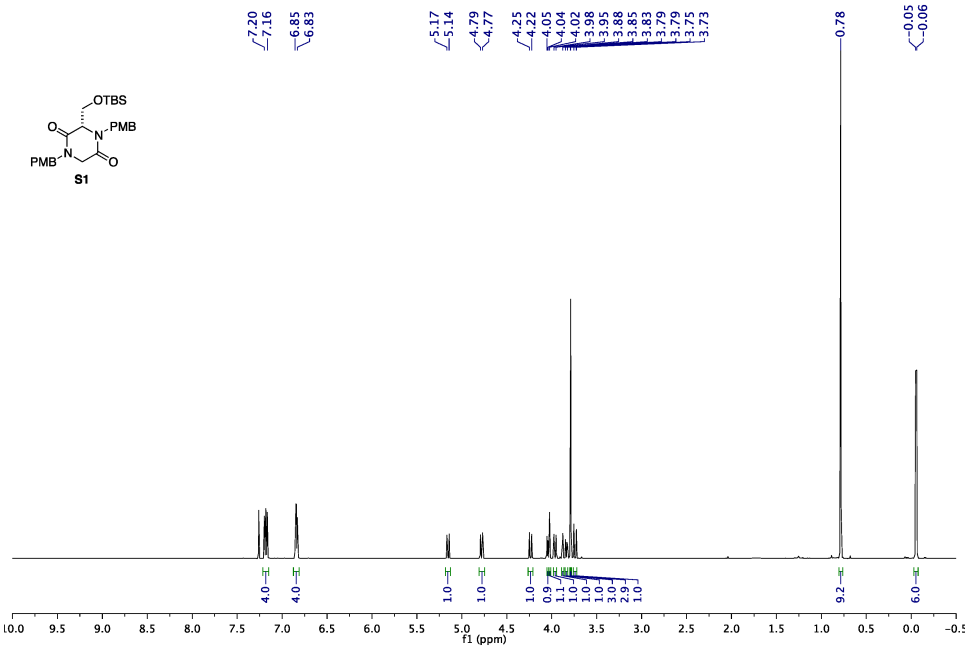
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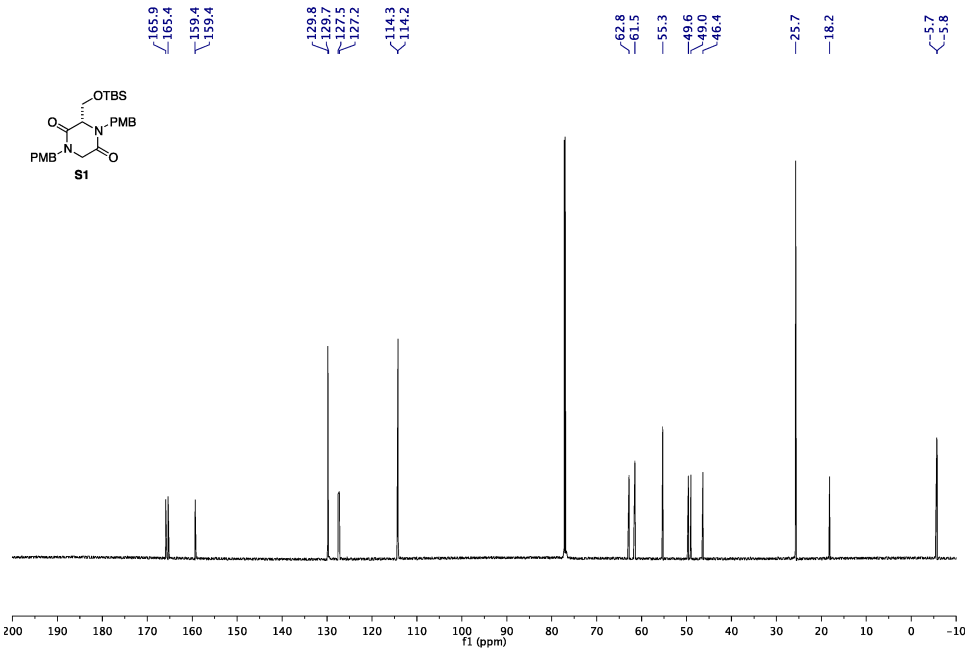
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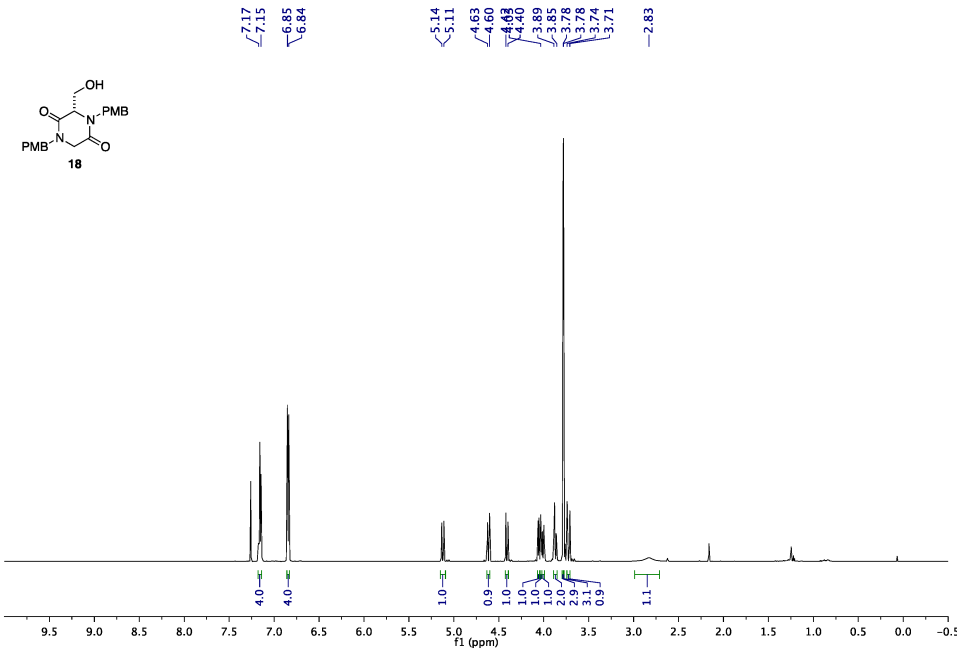
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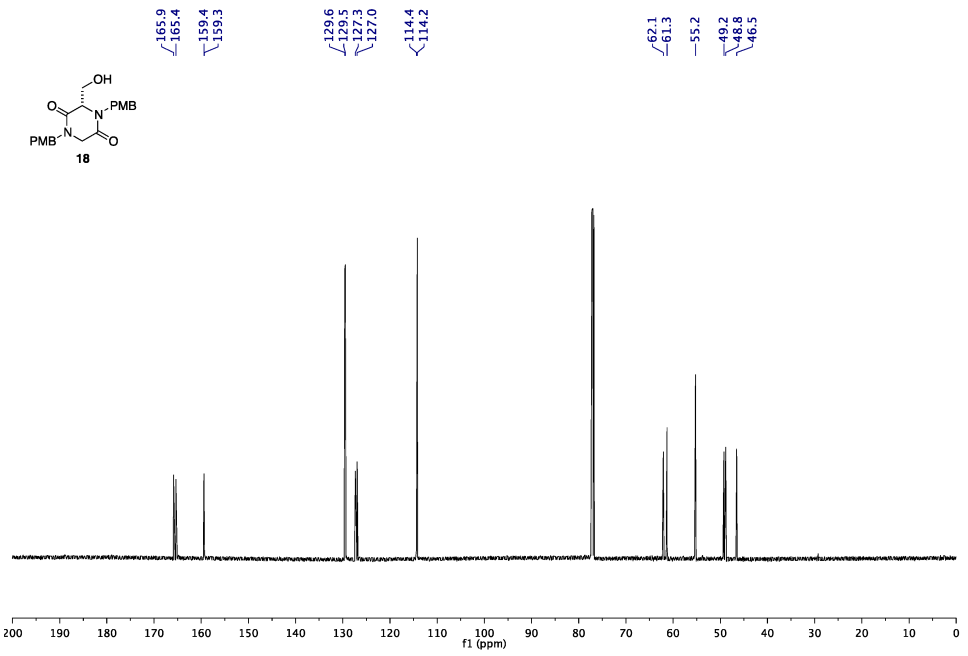
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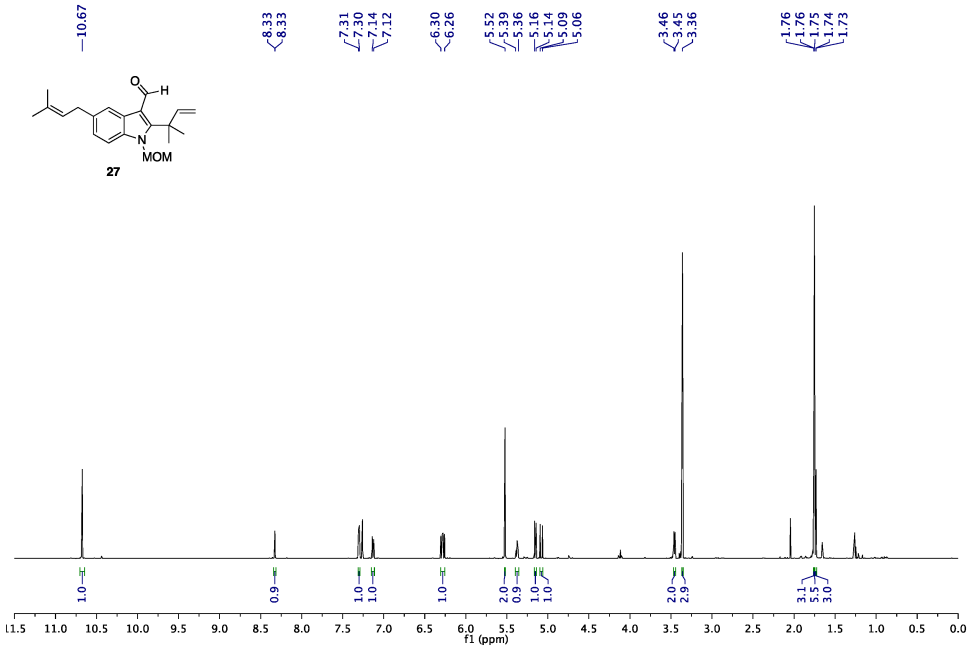
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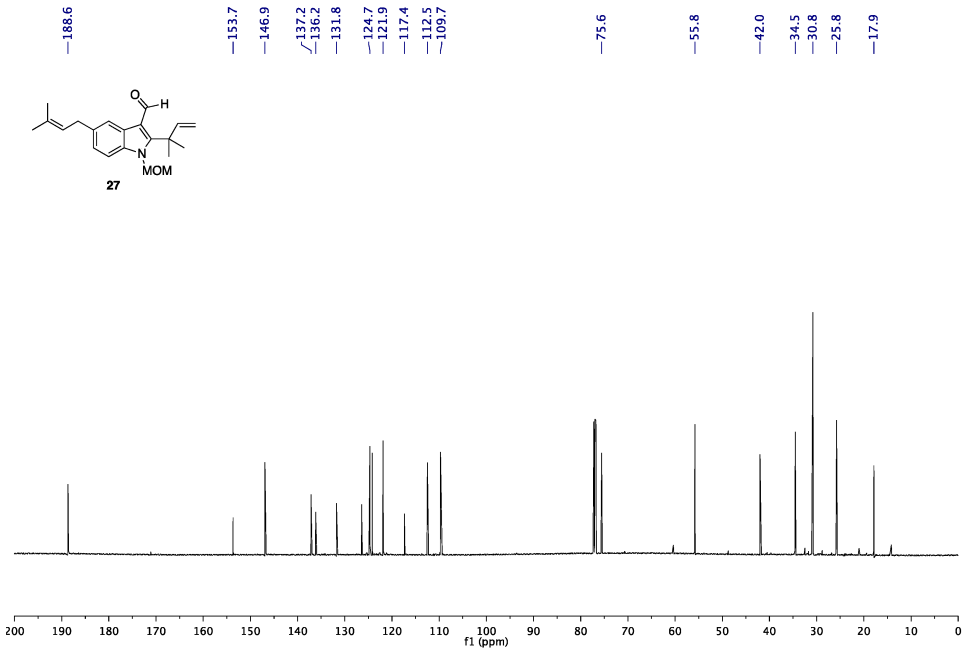
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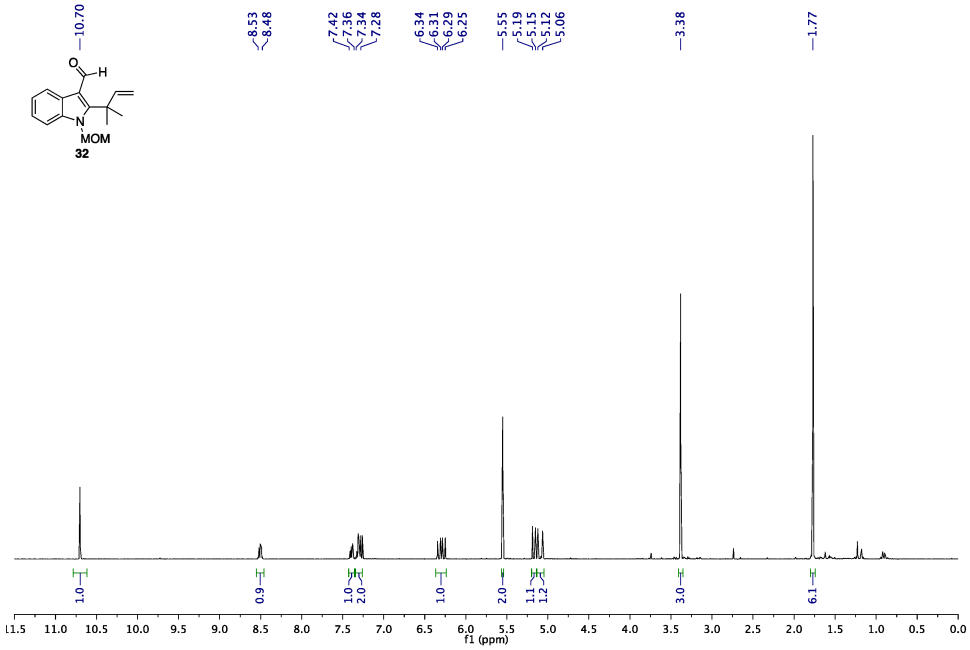
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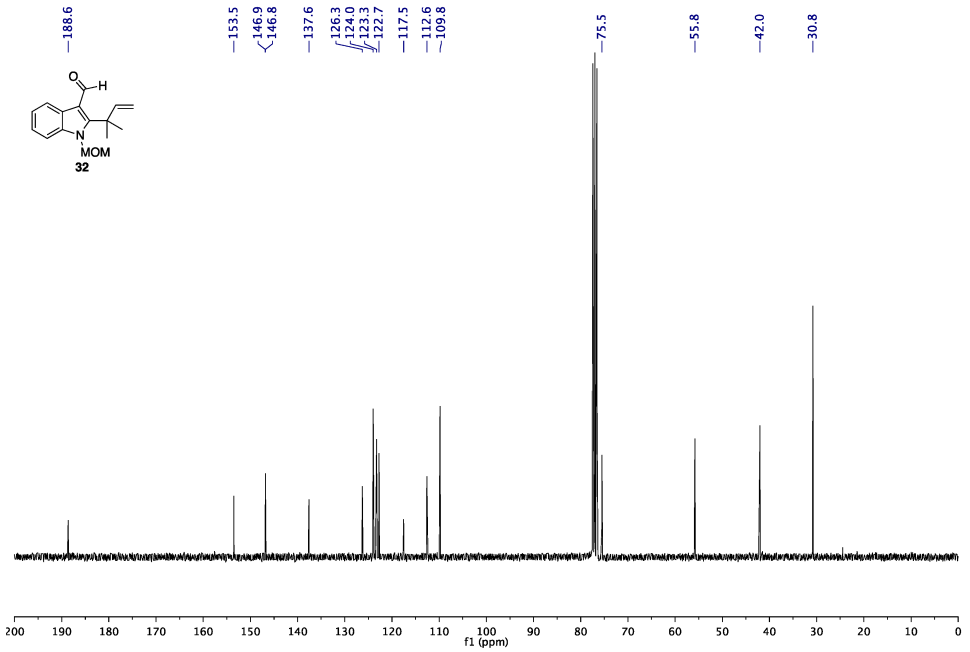
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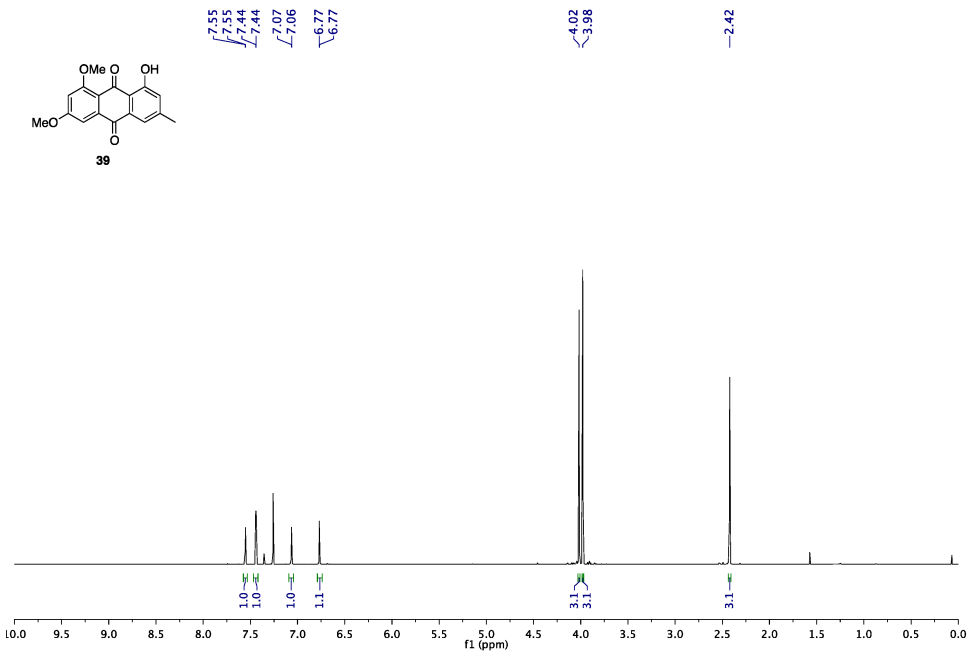
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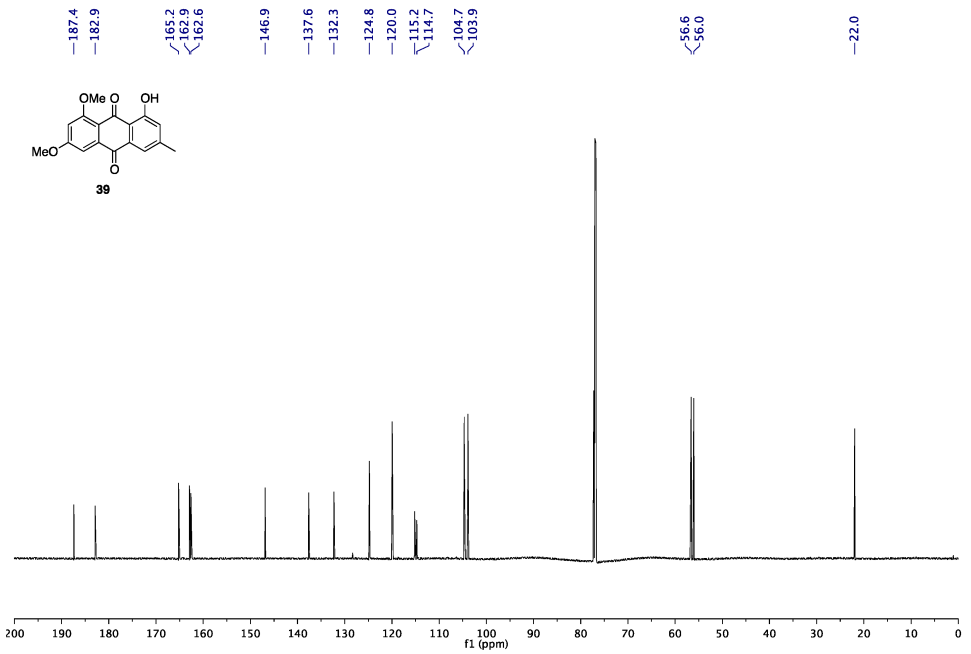
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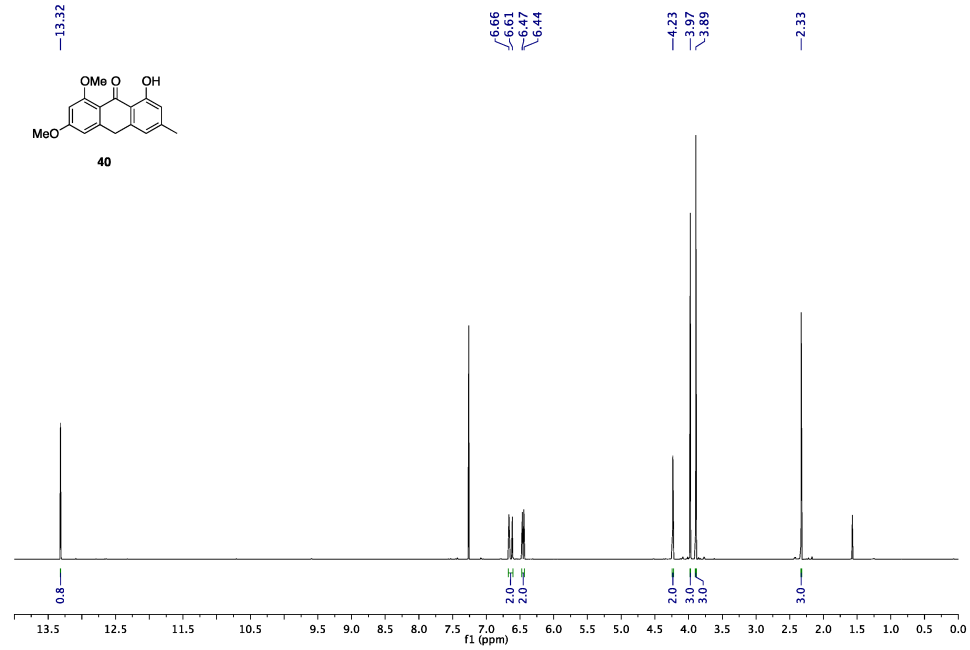
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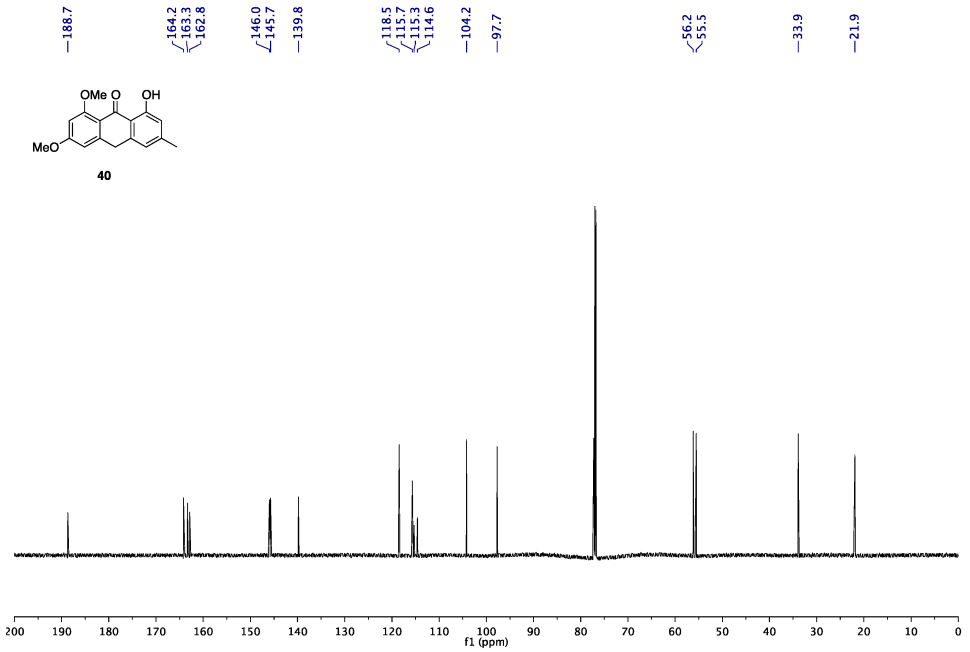
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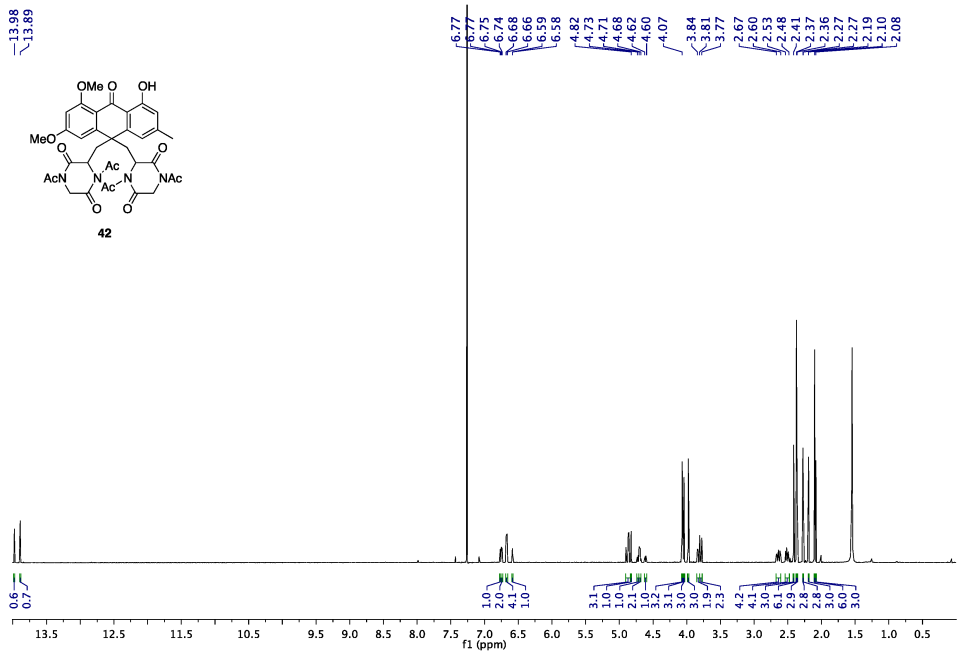
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4.68
4.62
4.60
4.07
3.84
3.81
3.77
2.67
2.60
2.53
2.48
2.41
2.37
2.36
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NAc
Ac
Ac
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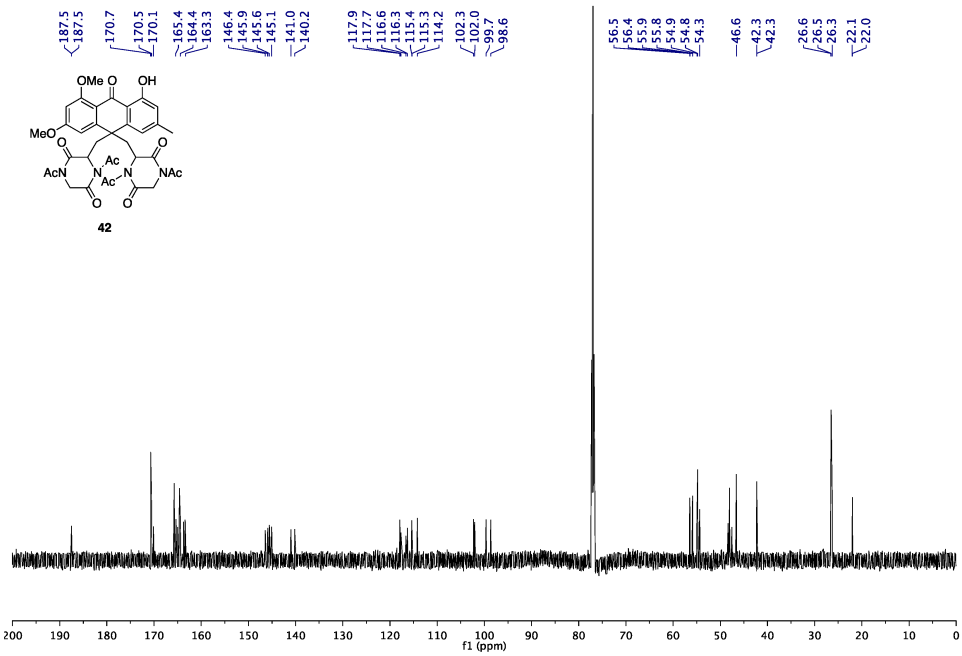
f1 (ppm)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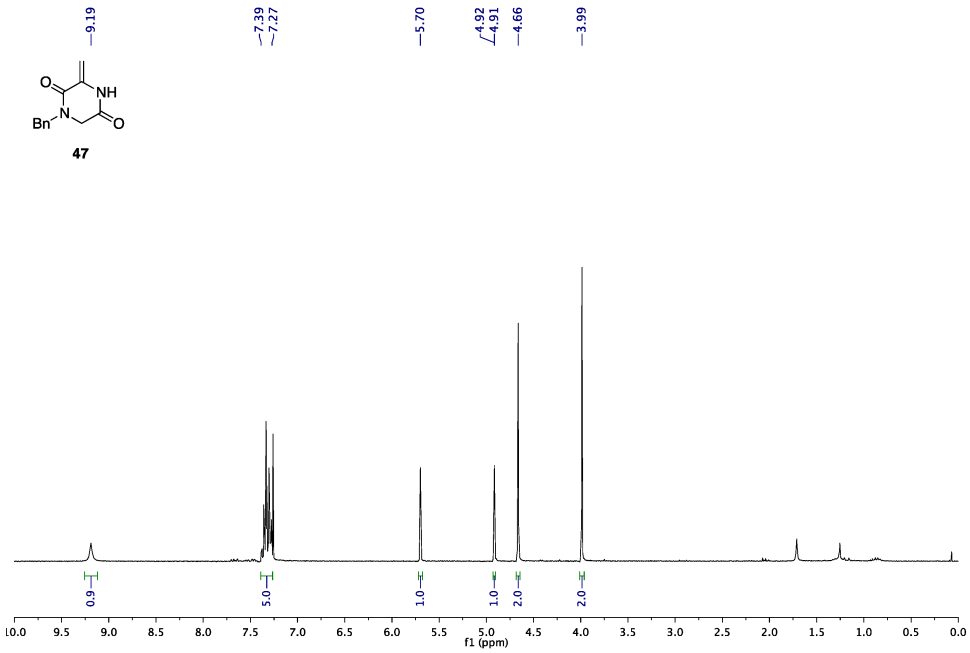
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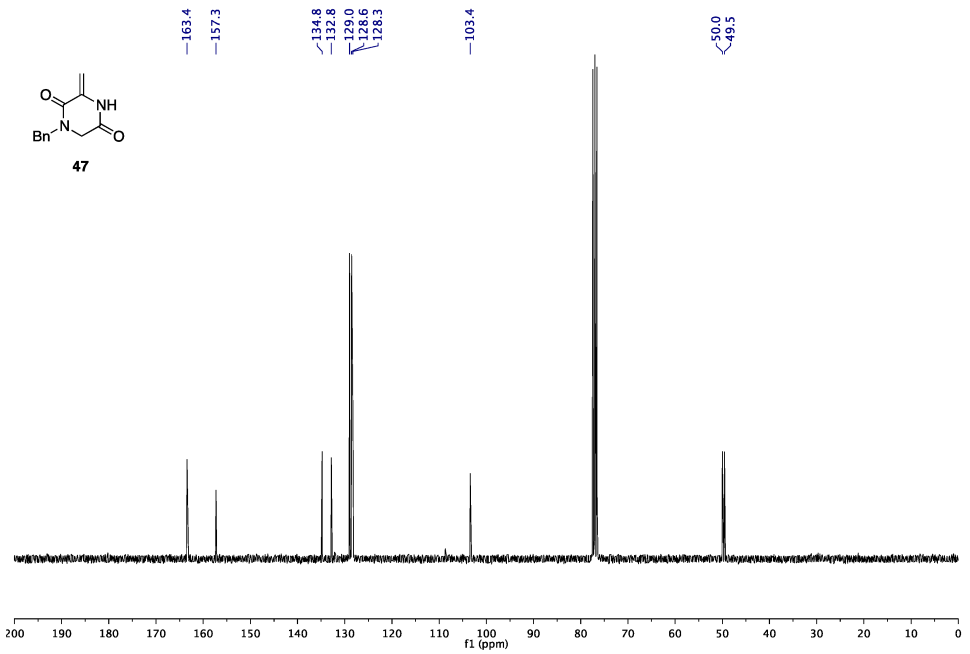
f1 (ppm)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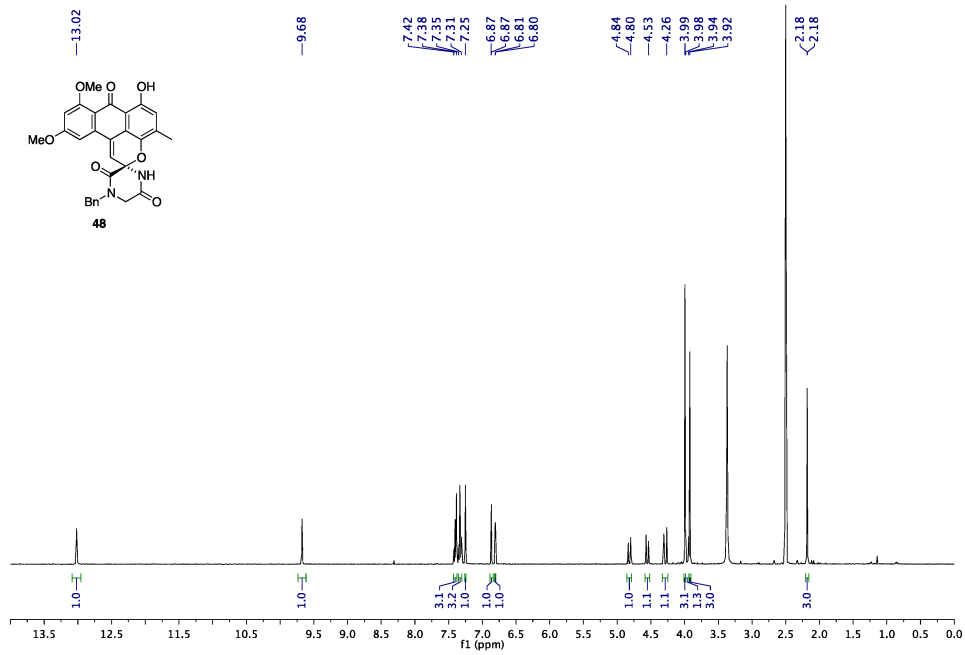
f1 (ppm)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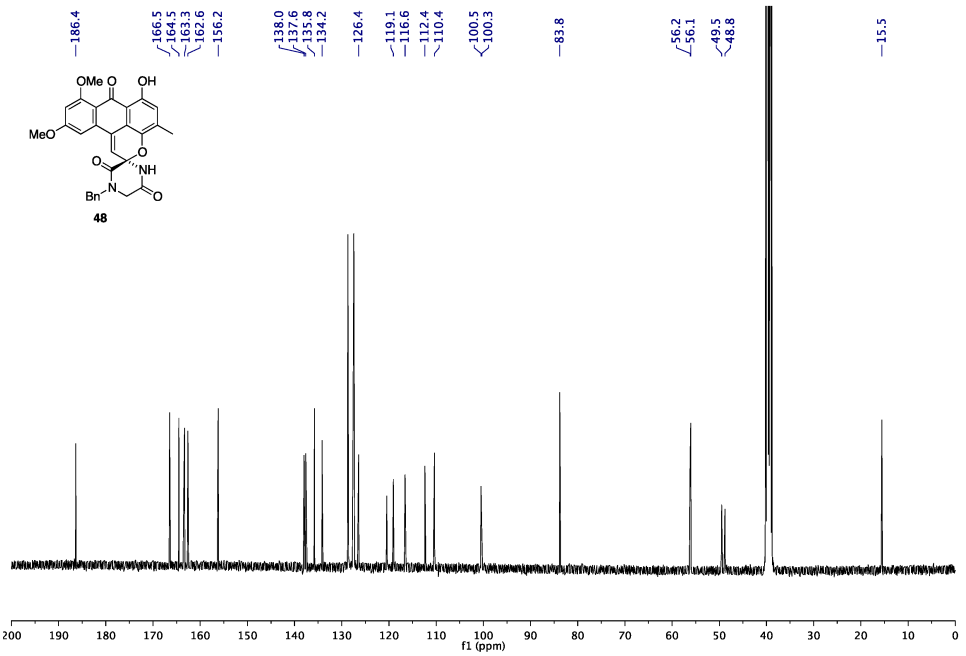
f1 (ppm)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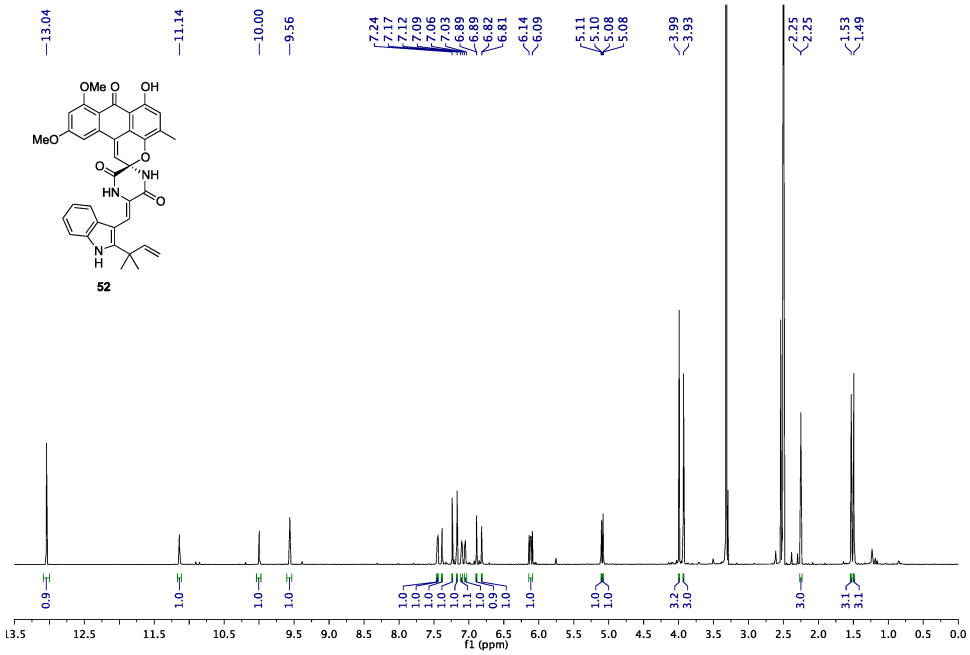
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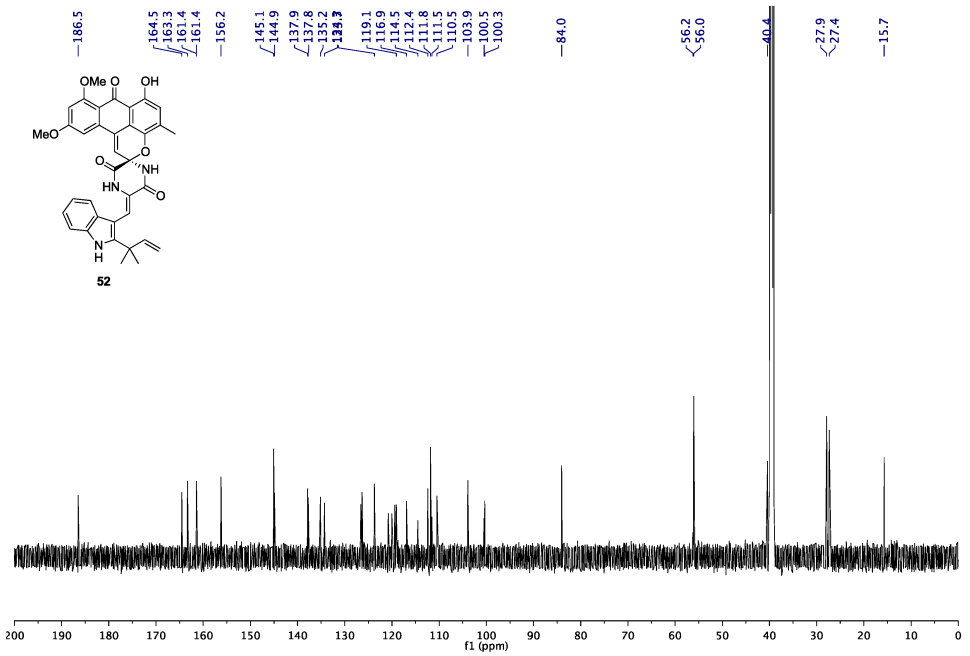
f1 (ppm)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HN
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H
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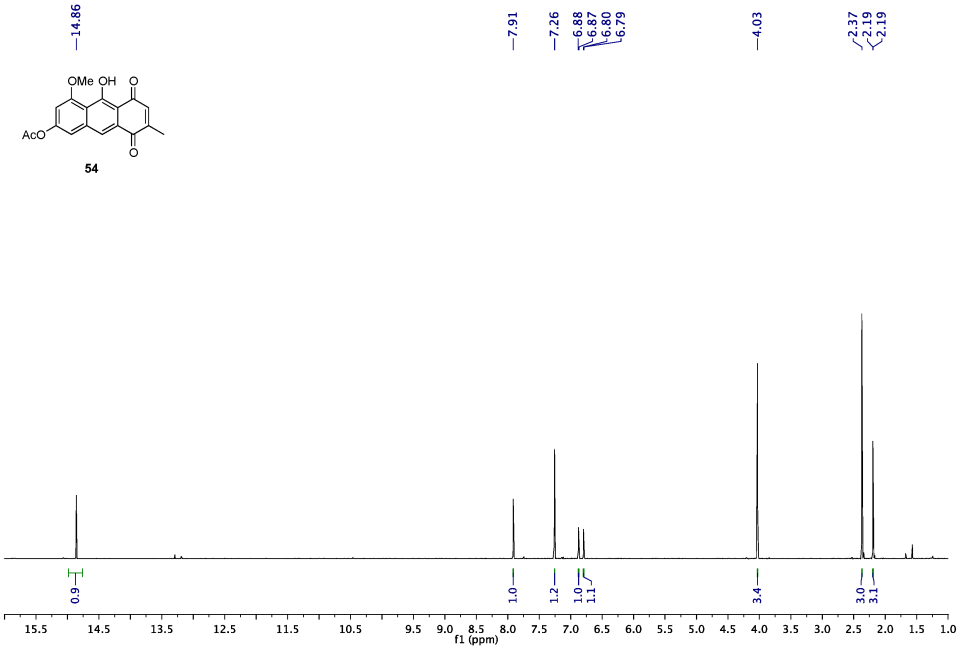
f1 (ppm)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AcO
O
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1.2
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1.1
3.4
3.0
3.1
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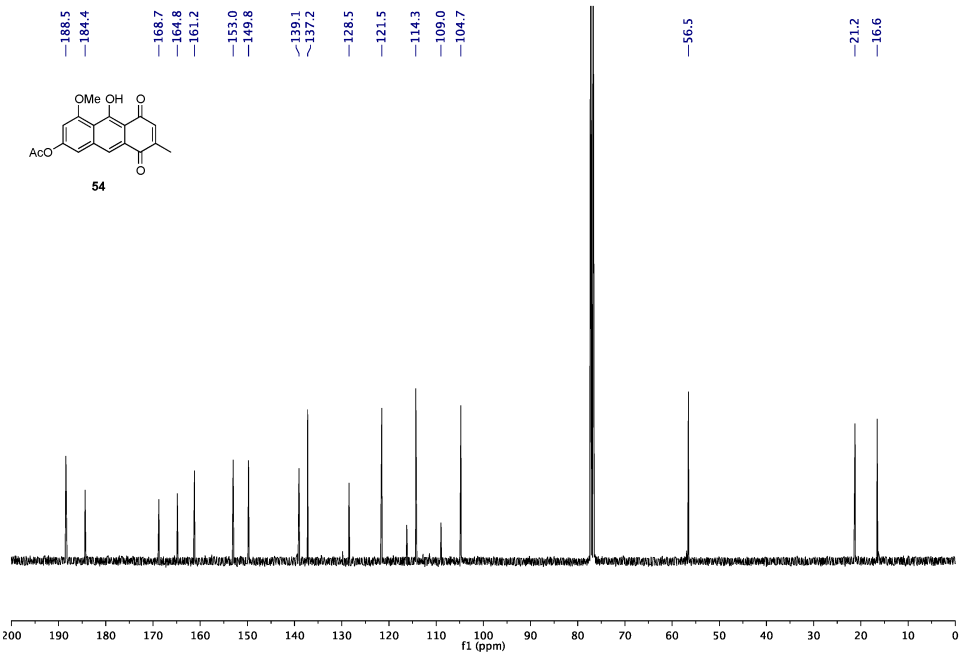
f1 (ppm)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104.7
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21.2
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AcO
O
54
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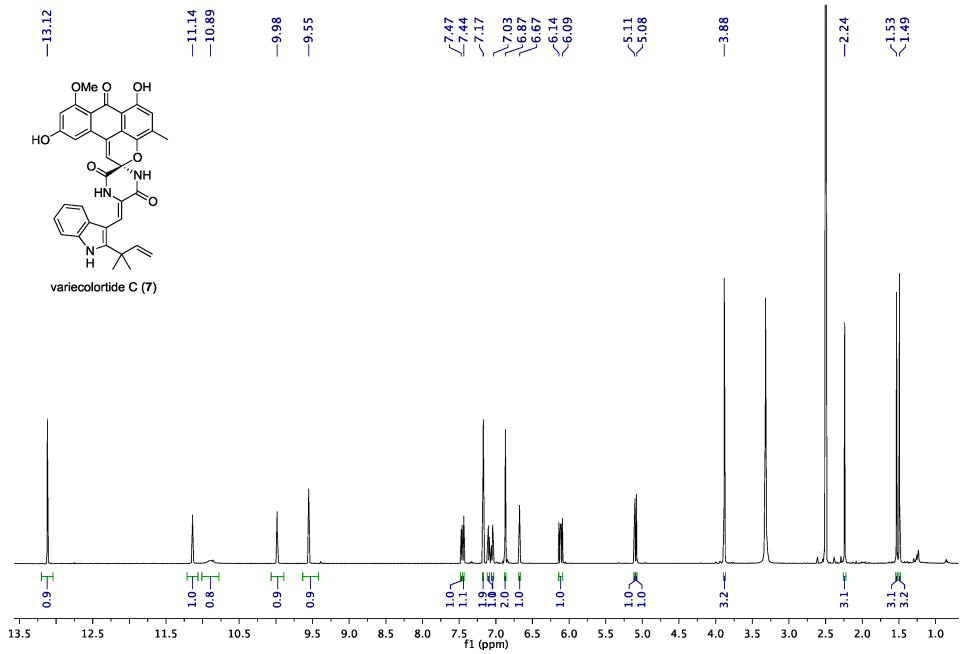
f1 (ppm)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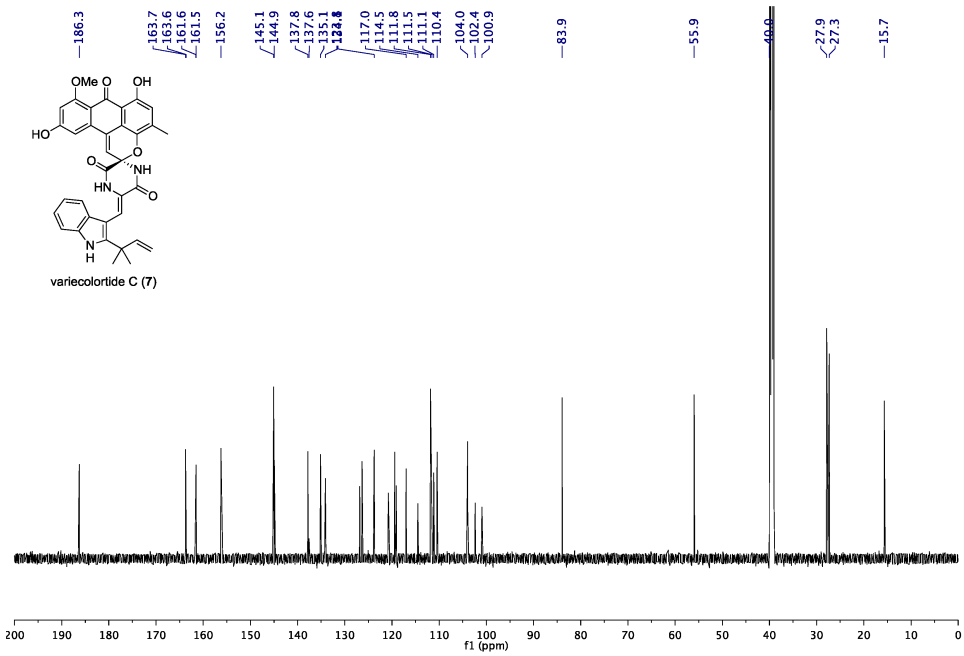
1.49
variecolortide C (7)
f1 (ppm)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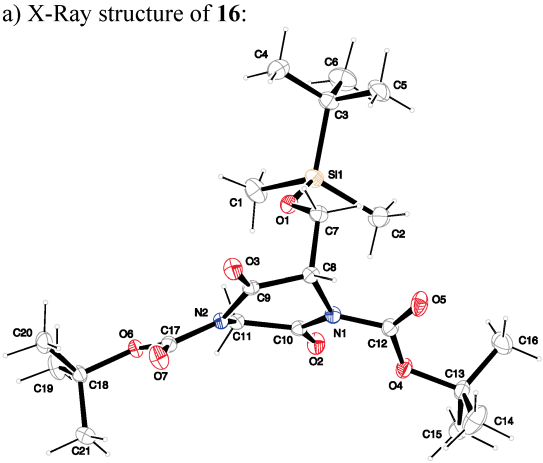
111.8
111.5
111.1
110.4
104.0
102.4
100.9
83.9
55.9
49.0
27.9
27.3
15.7
variecolortide C (7)
f1 (ppm)

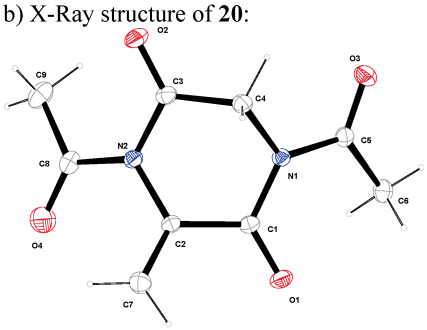
Crystal structures

Note: Crystallographic data for compounds **16**, **20**, **26**, **34** and **42** have been deposited at the Cambridge Crystallographic Data Centre (CCDC 883797, 883798, 883796, 883856, and 883799, respectively). No data has been deposited for compound **45** because of poor quality.

a) X-Ray structure of **16**:



b) X-Ray structure of **20**:



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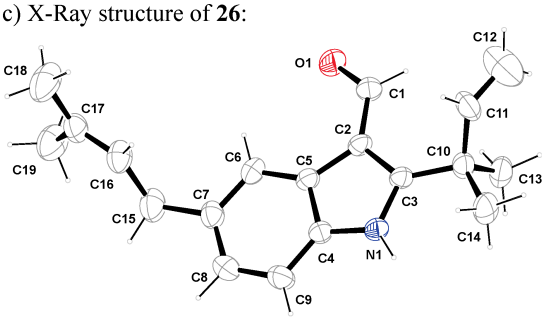
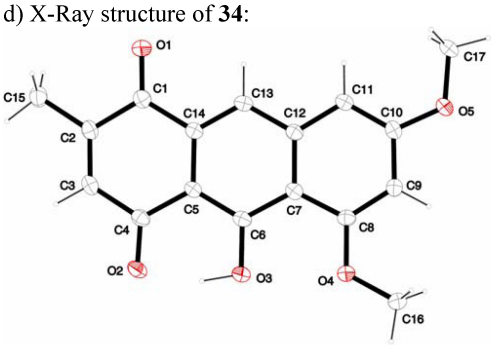
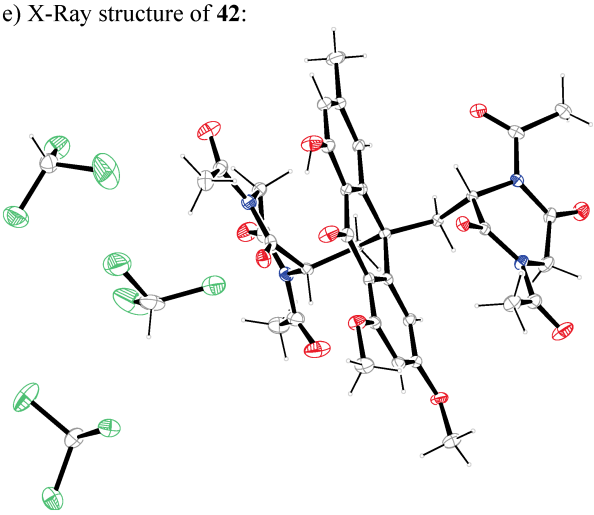


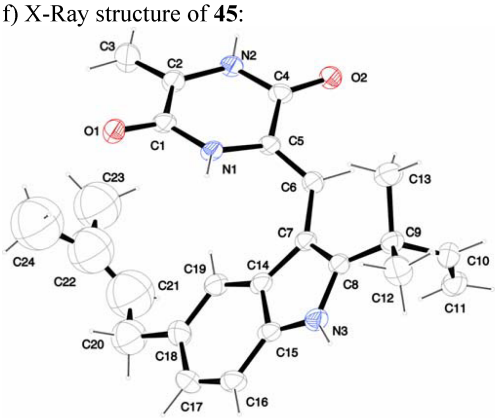
d) X-Ray structure of **34**:



e) X-Ray structure of **42**:



f) X-Ray structure of **45**:



Part II: Towards the Total Synthesis of Naphthomycin K and Divergolides C and D

1 Towards the Total Synthesis of Naphthomycin K

1.1 Introduction and Background

1.1.1 Ansamycins Antibiotics

Polyketides are natural products with great structural diversity and a wide range of biological activities.[1] The immunosuppressant rapamycin,[2] the antibiotic erythro-mycin A,[3] or the cytostatic drug doxorubicin[4] represent only a small selection of polyketides that play a crucial role in today´s medicine (Figure 1).

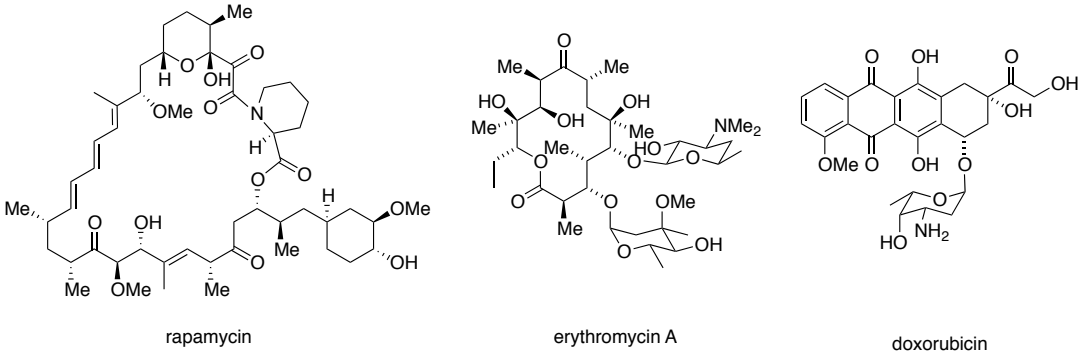


Figure 1. A selection of biologically active polyketide natural products.

One important class of polyketides that our group has become increasingly interested in are the so called ansamycins. These macrocyclic lactam antibiotics are characterized by their "basket-like" structures consisting of an aromatic moiety that is flanked at non-adjacent positions by an aliphatic chain, like a handle or "ansa" (lat. grip).[5] Lüttringhaus was the first who called this aliphatic moiety of varying length an *ansa* chain.[6] The term "ansamycins" was

[1] Dewick, P. M. *Medicinal Natural Products: A Biosynthetic Approach*, 3rd ed.; John Wiley & Sons Ltd: Chichester, 2009.
[2] (a) Vézina, C.; Kudelski, A.; Sehgal, S. N. *J. Antibiot.* **1975**, *28*, 721–726; (b) Sehgal, S. N.; Baker, H.; Vézina, C. *J. Antibiot.* **1975**, *28*, 727–732.
[3] McGuire, J. M.; Bunch, R. L.; Anderson, R. C.; Boaz, H. E.; Flyn, E. H.; Powell, H. M.; Smith, J. W. *Antibiot. Chemother.* **1952**, *2*, 281–283.
[4] Di Marco, A.; Gaetani, M.; Scarpinato B. *Cancer Chemother. Rep.* **1969**, *53*, 33–37.
[5] Wrona, I. E.; Agouridas, V.; Panek, J. S. *C. R. Chimie* **2008**, *11*, 1483–1522.
[6] Lüttringhaus, A.; Gralheer, H. *Liebigs Ann. Chem.* **1947**, *557*, 112–120.

later termed by Prelog and Oppolzer.[7] As depicted in Figure 2, the ansamycins can be divided into two groups based on their aromatic nucleus. The naphthalenoid or naphthalenic ansamycins (e.g. rifamycin, streptovaricin, naphthomycin) possess a 1,4-naphthoquinone or 1,4-hydroxy-naphthalene moiety as the chromophore. The benzenoid or benzenic ansamycins including e.g. geldanamycin, maytansine or herbimycin were isolated later than the corresponding naphthalenes and contain a 1,4-quinone or 1,4-hydroquinone unit. In terms of their biological activity, the naphthalenic ansamycins mostly exhibit strong antimicrobial activities,[8] whereas benzenic ansamycins have shown promise as potential antitumor agents.[9]

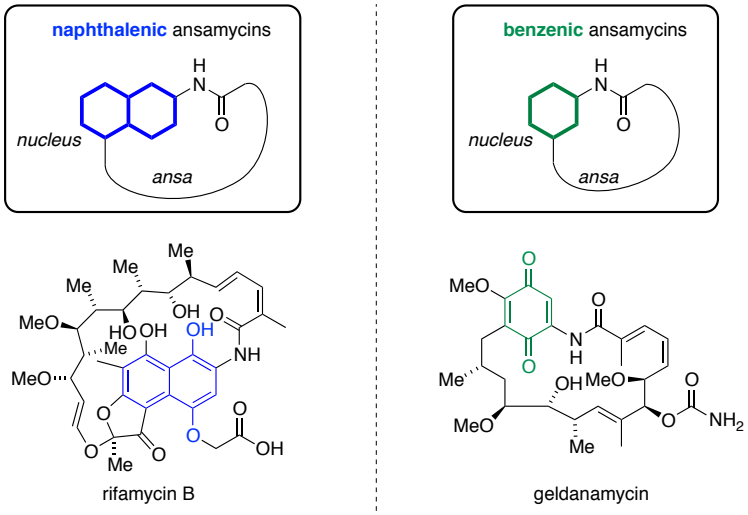


Figure 2. Classification of ansamycins based on their aromatic nucleus (illustrated in blue and green). Rifamycin B (a naphthalenic ansamycin) and geldanamycin (a benzenic ansamycin) are two examples of these groups of ansamycins.

Amongst the vast number of ansamycins that have been reported to date, we are particularly interested in naphthomycins A (**2.1**) and K (**2.2**), divergolides C (**2.3**) and D (**2.4**) and ansalactam (**2.5**), all of which are naphthalenic ansamycins (Figure 3). Due to their intriguing structures and interesting biological activities, we have engaged in their total syntheses. The major goal of the presented work in this chapter was the synthesis of a naphthalene core, which could be utilized in the synthesis of all of the targeted ansamycins.

In the following sections, a short background summary as well as the results of our recent efforts towards these ansamycins will be presented.

[7] Prelog, V.; Oppolzer, W. *Helv. Chim. Acta* **1973**, *56*, 2279–2287.
[8] Wehrli, W., Staehlin, M. *Bacteriol. Rev.* **1971**, *35*, 290–309.
[9] Cragg, G. M.; Kingston, D. G. I.; Newman, D. J. *Anticancer Agents from Natural Products,* 2nd ed.; Taylor & Francis: Boca Raton, 2012.

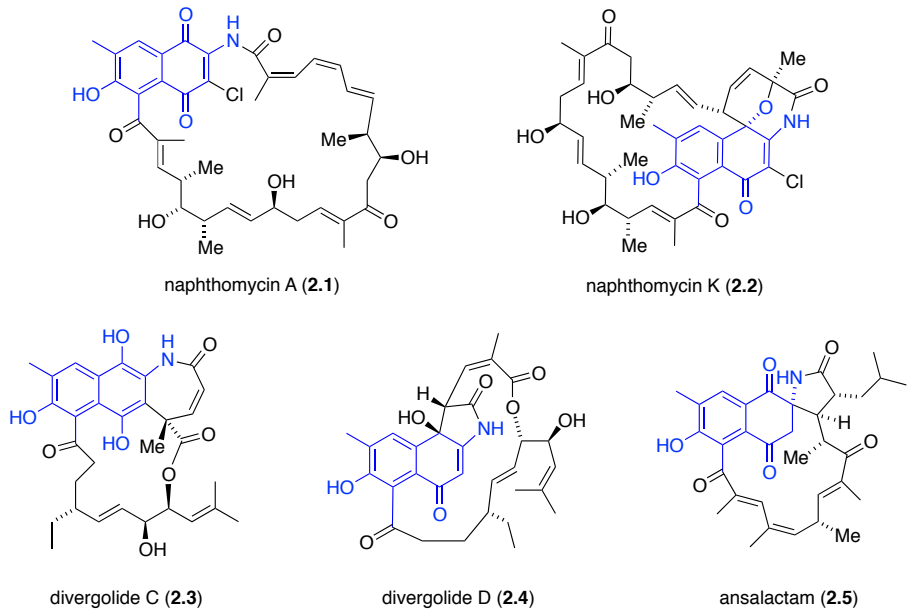


Figure 3. Naphthalenic ansamycins that were targeted as part of this Ph.D. thesis.

1.1.2 Naphthomycins A-J

The naphthomycins are a family of natural products that belong to the naphthalenoid ansamycins with C_{23} *ansa* chains. Their first member, naphthomycin A (**2.1**), was isolated in 1969 as a yellow pigment from the cultured broth of strain Tü 105 of *Streptomyces collinus* by Balerna *et al*.[10] It took 15 years until its absolute configuration could be elucidated, mainly by chemical degradation and X-ray structural analysis of a methylated derivative, 25-*O*-methylnaphthomycin A iminomethyl ether (**2.6**) (Figure 4).[11]

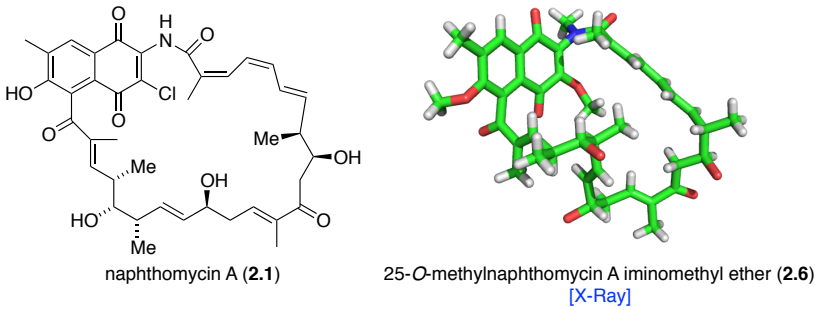


Figure 4. Structural drawing of naphthomycin A (**2.1**) and X-ray structure of its derivative 25-*O*-methyl-naphthomycin A iminomethyl ether (**2.6**).

As shown in Figure 5, further members of this family were subsequently isolated and reported. Naphthomycins B (**2.9**) and C (**2.10**) were isolated in 1983 from *S. collinus*, strain

[10] Balerna, M.; Keller-Schierlein, W.; Martius, C.; Wolf, H.; Zähner, H. *Arch. Mikrobiol.* **1969**, *65*, 303–317.
[11] Keller-Schierlein, W.; Meyer, M.; Cellai, L.; Cerrini, S.; Lamba, D.; Segre, A.; Fedeli, W.; Brufani, M. *J. Antibiot.* **1984**, *37*, 1357–1361.

Tü 353 and Tü 1892 respectively.[12] They differ from naphthomycin A not only in the configuration of their C4-C5 double bond, but also lack a methyl group at C2 (Figure 5). Three years later, the isolation of four other naphthomycins, namely naphthomycins D (**2.7**), E (**2.8**), F (**2.11**) and G (**2.12**), from the strain Tü 2357 of *Streptomyces aurantiogriseus*, was reported by the same group.[13] Distinguishing features of naphthomycins D (**2.7**) and E (**2.8**) are an OH and H substituent at C30 instead of a Cl substituent present in naphthomycin A (**2.1**). Naphthomycins F (**2.11**) and G (**2.12**) on the other hand, possess an *N*-acetylcysteine residue linked to C30 of the naphthoquinoid moiety by a thioether group. Whilst naphthomycin F (**2.11**) displays some biological activity against gram-positive bacteria and fungi, naphthomycins D (**2.7**), E (**2.8**) and G (**2.12**) were found to be inactive against microorganisms. The structure of naphthomycin H (**2.13**)[14] was published by Mukhopadhyay *et al.* in 1985 and finally, Hooper and coworkers reported the isolation of naphthomycins I (**2.12**, identical to napthomycin G) and J (**2.14**).[15]

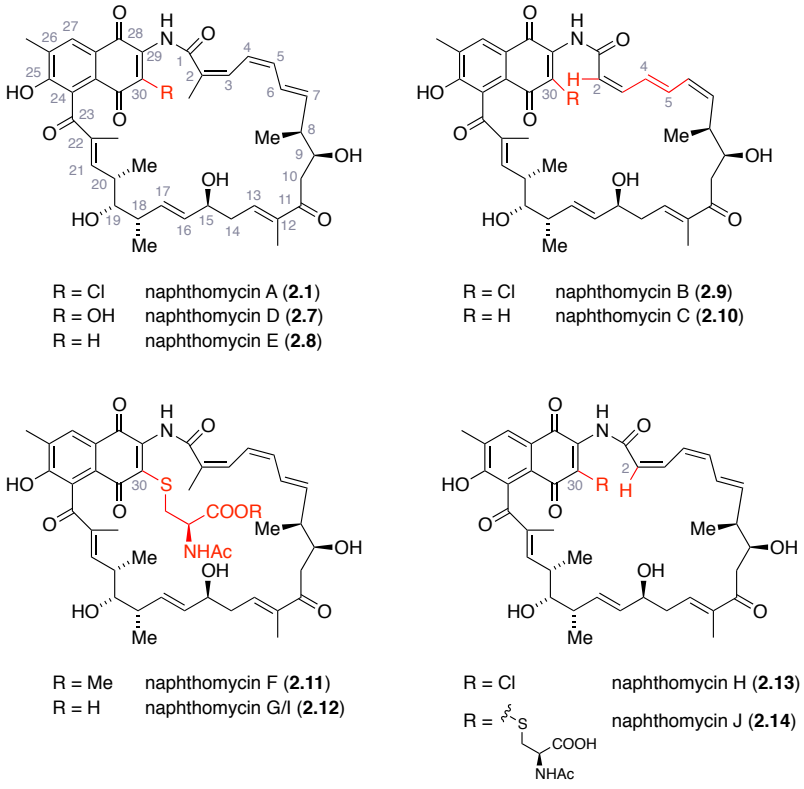


Figure 5. Structures of naphthomycins A–J. Differences from the parent naphthomycin A (**2.1**) are highlighted in red.

[12] Keller-Schierlein, W.; Meyer, M.; Zeeck, A.; Damberg, M.; Machinek, R.; Zähner, H.; Lazar, G. *J. Antibiot.* **1983**, *36*, 484–492.
[13] Meyer, M.; Keller-Schierlein, W.; Megahed, S.; Zähner, H.; Segre, A. *Helv. Chim. Acta* **1986**, *69*, 1356–1364.
[14] Mukhopadhyay, T.; Franco, C. M. M.; Reddy, G. C. S.; Ganguli, B. N.; Fehlhaber, H. W.; *J. Antibiot.* **1985**, *38*, 948–951.
[15] Hooper, A. M.; Rickards, R. W. *J. Antibiot.* **1998**, *51*, 845–851.

1.1.3 Naphthomycin K

The newest member of the naphthomycin family is naphthomycin K (**2.2**), which was reported in 2007 by Lu and Shen.[16] Naphthomycin K (**2.2**) was isolated together with naphthomycins A (**2.1**) and E (**2.8**) from the commercial strain *Streptomyces* sp. CS of the medicinal plant *Maytenus hookeri* (Figure 6). Its structure was elucidated by the analysis of NMR and MS data. Interestingly, whilst naphthomycins are usually known for their antibacterial and antifungal activities, **2.2** displayed cytotoxic activity against P388 and A-549 cell lines.

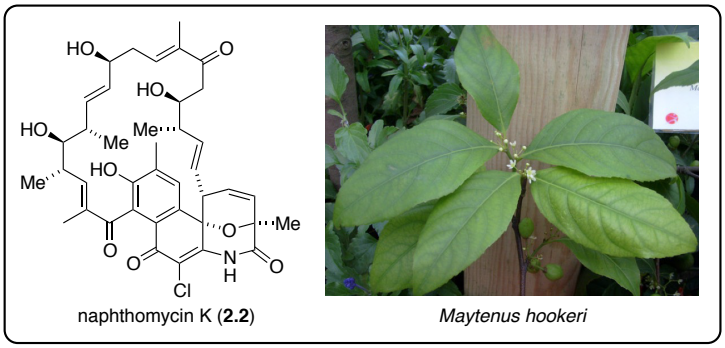


Figure 6. Structure and plant source[17] of the natural product naphthomycin K (**2.2**).

In addition to its interesting biological activity, we were particularly attracted by the unusual structure of naphthomycin K (**2.2**). It features nine stereogenic centers, a highly functionalized *ansa* chain and an unprecedented naphthoquinone core that contains a substituted oxa-aza-bicyclo[3.3.1]-nonenone ring system. What we found particularly interesting is the fact that the published structure of naphthomycin K (**2.15**) differs from the structures of naphthomycin A–J in two important aspects (Figure 7). In the structure of naphthomycin K (**2.15**) assigned by Lu and Shen, the absolute configuration of the methyl group at C8 is (*R*). Furthermore, the double bond geometry between C21 and C22 is claimed to be (*Z*). However, all other naphthomycins that have been reported to date share a methyl group at C8 that has a (*S*)-configuration and a (*E*) double bond between C21 and C22. We thus believe that the reported structure might be misassigned and herein propose a slightly different structure of naphthomycin K (**2.2**), which we wish to prove by total synthesis.

[16] Lu, C.; Shen, Y. *J. Antibiot.* **2007**, *60*, 649–653.
[17] Source of picture: http://tupian.hudong.com

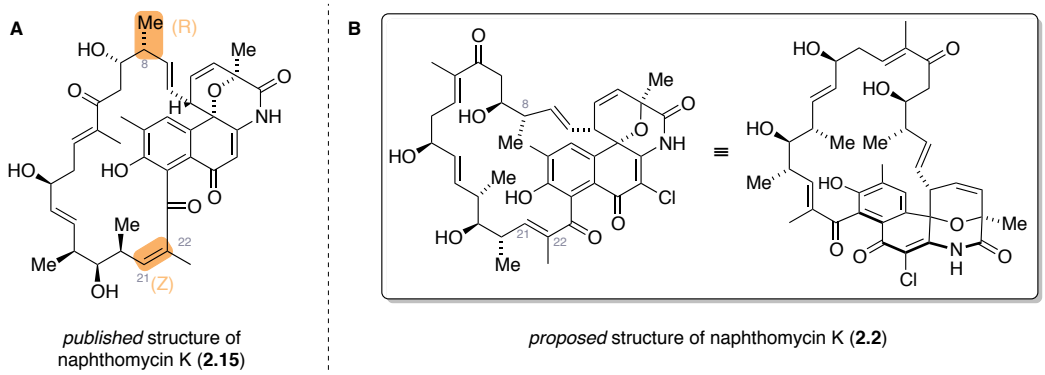
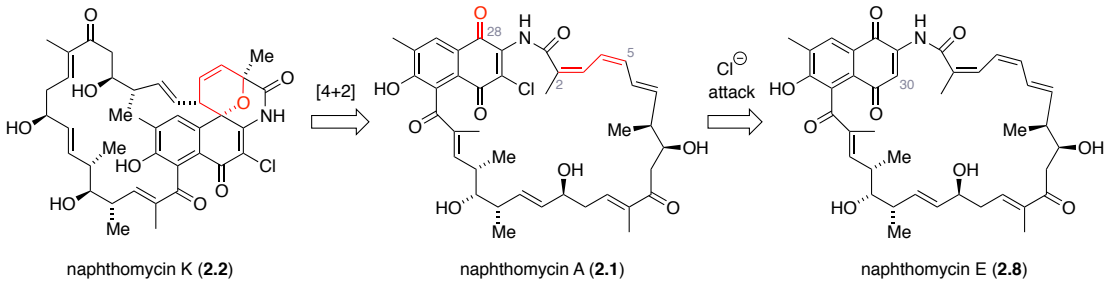


Figure 7. **A**. Structure of naphthomycin K (**2.15**) assigned by Lu and Shen.[16] **B**. Structure of naphthomyin K (**2.2**) proposed by our group.

1.1.4 Biosynthesis of the Naphthomycins

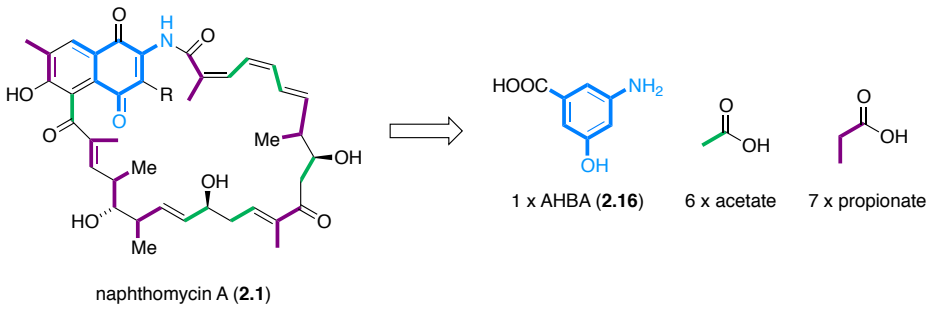
As depicted in Scheme 1, we believe that naphthomycin K (**2.2**) is formed from naphthomycin A (**2.1**) *via* hetero-Diels-Alder reaction between the carbonyl group at C28 and the diene between C2 and C5. Naphthomycin A (**2.1**) in turn, could be formed from naphthomycin E (**2.8**) by chlorination at C30. Our assumption that **2.1** and **2.8** are biosynthetic precursors of naphthomycin K (**2.2**) is supported by the fact that naphthomycins A (**2.1**) and E (**2.8**) were isolated together with naphthomycin K (**2.2**) from the same organism.



Scheme 1. Proposed formation of naphthomycin K (**2.2**) from its biosynthetic precursors naphthomycin A (**2.1**) and E (**2.8**).

The biosynthesis of naphthomycin A (**2.1**) was studied by Lee and coworkers and published in 1994.[18] It was shown by ^{13}C-labeling experiments and NMR-analysis that **2.1** is assembled *via* the polyketide pathway and that its structure can be traced back to 3-amino-5-hydroxybenzoic acid (AHBA, **2.16**) as the starter unit (mC_7N unit), seven propionate (methyl-malonyl-CoA) and six acetate (malonyl-CoA) chain extension units (Scheme 2).

[18] Lee, J. P.; Tsao, S.-W.; Chang, C.-J.; He, X.-G.; Floss, H. G. *Can. J. Chem.* **1994**, *72*, 182–187.



Scheme 2. Biosynthetic analysis of naphthomycin A (**2.1**). It is derived from one mC_7N, six acetate and seven propionate units.

AHBA is synthesized *via* the aminoshikimate pathway from 5-deoxy-5-amino-3-dehydroshikimic acid (amino-DHS) by the action of AHBA synthase.[19]

Recently, Wu *et al.* reported the cloning and functional analysis of the naphthomycin biosynthetic gene cluster in *Streptomyces* sp. CS.[20] Deduced from their results, the biosynthesis of naphthomycin A is initiated by a AHBA starter unit which is extended to a tetraketide by incorporation of two methyl-malonyl-CoA and one malonyl-CoA unit (Figure 8).

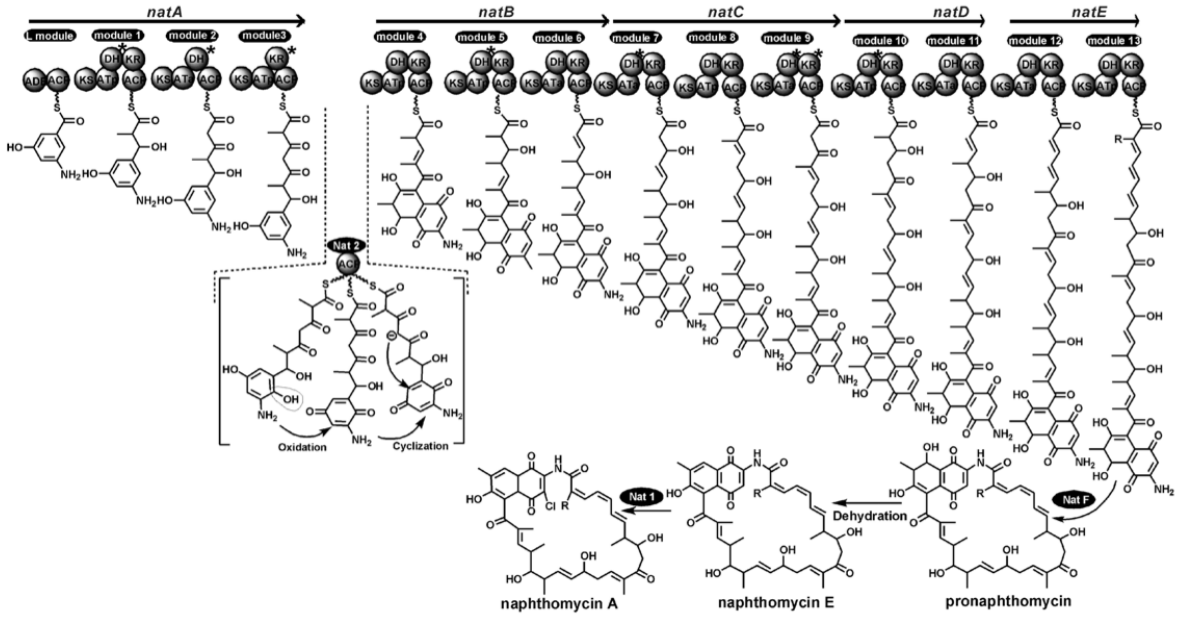


Figure 8. Putative biosynthetic pathway for naphthomycin A. ADE, carboxylic acid: ACP ligase (loading domain); KS, β-ketoacyl-ACP synthase; DH, β-hydroxyacyl-thioester dehydratase; KR, β-ketoacyl-ACP reductase; ER, enoyl, reductase. The putative intermediates in chain-extension cycles and the *nat* genes involved in the various biosynthetic steps are indicated. The redundant domains are labeled with *. R, methyl or H.[20] Source: [20]

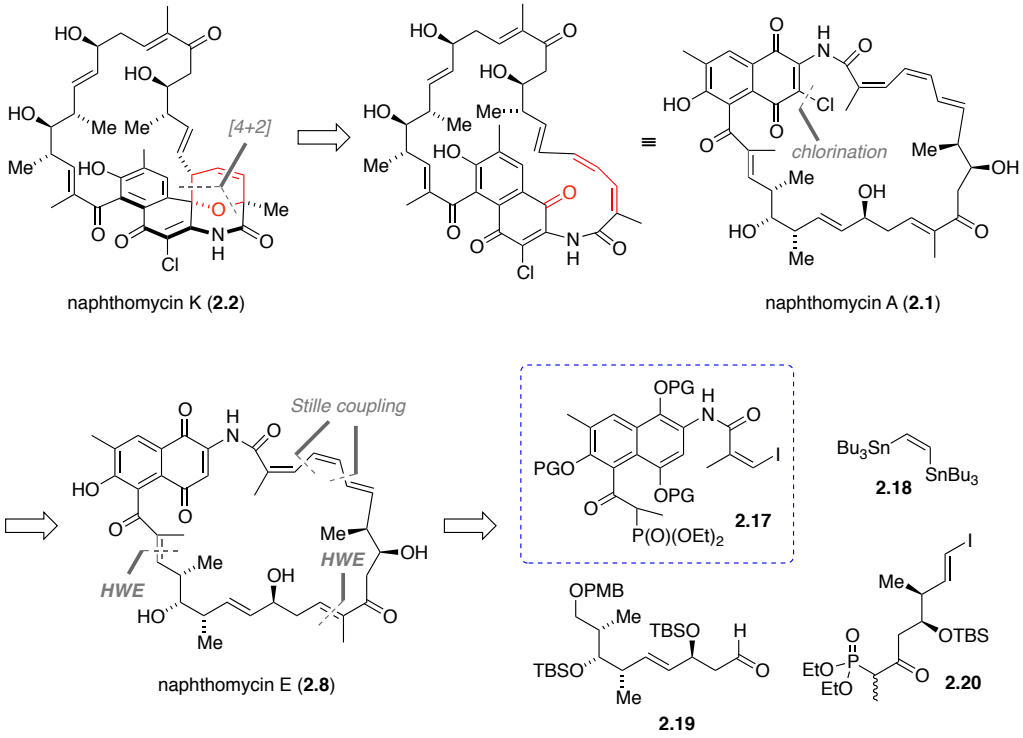
[19] (a) Kim, C.-G.; Kirschning, A.; Bergon, P.; Ahn, Y.; Wang, J. J.; Shibuya, M.; Floss, H. G. *J. Am. Chem. Soc.* **1992**, *114*, 4941–4943; (b) Kim, C.-G.; Kirschning, A.; Bergon, P.; Zhou, P.; Su, E.; Sauerbrei, B.; Ning, S.; Ahn, Y.; Breuer, M.; Leistner, E.; Floss, H. G. *J. Am. Chem. Soc.* **1996**, *118*, 7486–7491; (c) Kim. C.-G.; Yu, T.-W.; Fryhle, C. B.; Handa, S.; Floss, H. G. **1998**, *273,* 6030–6040.

[20] Wu, Y.; Kang, Q.; Shen, Y.; Su, W.; Bai, L. *Mol. BioSyst.* **2011**, *7*, 2459–2469.

Nat2 mediated oxidation of this tetraketide intermediate followed by cyclization gives rise to the naphthalene ring. This intermediate is further extended with five methyl-malonyl-CoA and five malonyl-CoA units to produce a linear polyketide chain. The nascent chain is then transferred, hydrolyzed and cyclized by NatF to form the 29-membered pronaphthomycin. The latter is dehydrated to form naphthomycin E (**2.8**). Finally, **2.8** is converted to naphthomycin A (**2.1**) by chlorination of C-30. Wu and coworkers were able to show that the halogenase gene *nat1* is responsible for this final step.[20]

1.1.5 Retrosynthetic Analysis of Naphthomycin K

In order to prove if our proposed structure of naphthomycin K was correct and to provide material in sufficient quantities to allow for its further biological evaluation, we engaged in the total synthesis of **2.2**. Our retrosynthesis, which is depicted in Scheme 3, was influenced by several considerations: 1) it should provide access to naphthomycins A (**2.1**) and E (**2.8**), so that we could test our biosynthetic hypothesis and 2) it should be convergent enough to target all members of the naphthomycin family by minor changes in the overall strategy. As such we expected naphthomycin K (**2.2**) to be formed from naphthomycin A (**2.1**) by an intramolecular hetero-Diels-Alder reaction. The latter would arise from naphthomycin E (**2.8**) by introduction of a chlorine substituent at C30. Naphthomycin E (**2.8**) is further dissected into four building blocks: naphthalene **2.17**, stannane **2.18**, phosphonate **2.19** and aldehyde **2.20**. While the linkage of building blocks **2.17**, **2.19** and **2.20** should be feasible by Horner-Wadsworth-Emmons (HWE) reaction, we envisioned that a double Stille-coupling would facilitate late-stage macrocyclization. This “double Stille stitching approach” would not only allow us to construct the challenging triene system but also would provide access to other naphthomycins when using the *E*-isomer of **2.18** and a naphthalene bearing a hydrogen instead of a methyl group at C2.

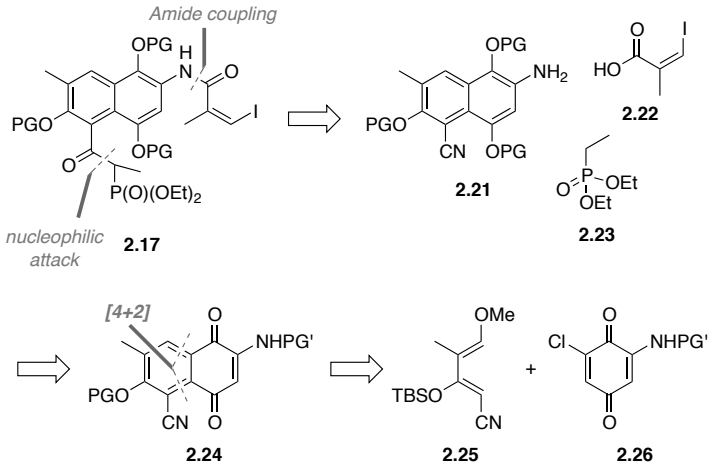


Scheme 3. Retrosynthetic analysis of naphthomycin K (**2.2**).

From the very beginning, this project was a very fruitful collaboration between Ph.D. student Mesut Cakmak and myself. While Mesut Cakmak was responsible for the synthesis of the *ansa* chain of naphthomycin K, the goal of my work was to develop a scalable route to a unified naphthalene building block, which could be used for the synthesis of not only the naphthomycins, but also for the divergolides and ansalactam.

1.1.6 1st Approach to the Naphthalene Core

Our initial approach towards naphthalene building block **2.17** is depicted in Scheme 4 in a retrosynthetic format. As such, **2.17** would be excised to reveal cyano-naphthalene **2.21**, β-iodomethacrylic acid **2.22** and commercially available phosphonate **2.23**. The linkage between **2.21** and phosphonate **2.23** was planned to be formed by nucleophilic attack of lithiated **2.23** at the cyano substituent of **2.21** and subsequent hydrolysis. The vinyl iodide **2.22** would in turn be connected to the naphthalene core **2.21** by simple amide coupling. Finally, the suitably protected naphthalene core was expected to be formed by Diels-Alder reaction between a suitable quinone **2.26** and the novel cyano-substituted diene **2.25**, followed by reduction and protection.



Scheme 4. 1st retrosynthetic analysis of naphthalene building block **2.17**.

1.2 Published Results

1.2.1 An Approach to Aminonaphthoquinone Ansamycins Using a Modified Danishefsky Diene

Publication: Kuttruff, C. A.; Geiger, S.; Cakmak, M.; Mayer, P.; Trauner, D. *Org. Lett.* **2012**, *14*, 1070–1073.

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An Approach to Aminonaphthoquinone Ansamycins Using a Modified Danishefsky Diene

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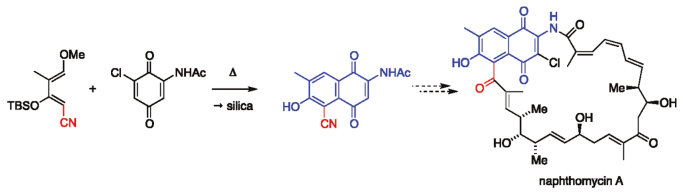
ABSTRACT



A robust and scalable synthesis of a novel, cyano-substituted Danishefsky-type diene and its use in the Diels–Alder reaction with various dienophiles is reported. The diene allows for the rapid construction of highly substituted aminonaphthoquinones that occur in numerous ansamycin antibiotics.

Ansamycins are an important class of natural products that show potent antibacterial and antiviral activities. In addition to members of the family that have long been known, such as rifamycin or naphthomycin A (**1a**),[1] several new aminonaphthoquinone ansamycins with intriguing structures have recently been reported, including naphthomycin K (**1b**),[2] ansalactam (**2**),[3] and divergolides C (**3a**) and D (**3b**).[4] As depicted in Figure 1, these molecules possess structurally diverse *ansa* chains of varying lengths that are mounted to a shared naphthoquinone core (depicted in blue) through an acyl linkage in position 5 and an amide in position 2 (naphthoquinone nomenclature). Several members have additional C–C bonds between the aromatic core and the *ansa* chain, which is remarkable from both a synthetic and biosynthetic point of view.

Our interest in the total synthesis of these natural products prompted us to devise a unified approach to their aminonaphthoquinone core (Scheme 1). We reasoned that due to steric compression, attachment of an *ansa* chain to the arene would be a challenge. This led us to consider cyano naphthalene **4** as a key intermediate, which in turn could be traced back to cyano-substituted Danishefsky diene **5** and substituted aminoquinone **6** *via* Diels–Alder reaction.

The original Danishefsky diene[5] has been widely used in organic synthesis along with several variations, which have been developed to improve its reactivity and synthetic scope.[6] These include alterations of the electron-donating substituents in positions 1 and 3, as well as the introduction of further substituents in positions 2 and 4 (diene nomenclature) that are not lost following cycloaddition.[7] However, to the best of our knowledge, there is little, if any,

(1) Keller-Schierlein, W.; Meyer, M.; Cellai, L.; Cerrini, S.; Lamba, D.; Segre, A.; Fedeli, W.; Brufani, M. *J. Antibiot.* **1984**, *37*, 1357–1361.
(2) Lu, C.; Shen, Y. *J. Antibiot.* **2007**, *60*, 649–653.
(3) Wilson, M. C.; Nam, S.-J.; Gulder, T. A. M.; Kauffman, C. A.; Jensen, P. R.; Fenical, W.; Moore, B. S. *J. Am. Chem. Soc.* **2011**, *133*, 1971–1977.
(4) Ding, L.; Maier, A.; Fiebig, H.-H.; Goerls, H.; Lin, W.-H.; Peschel, G.; Hertweck, C. *Angew. Chem., Int. Ed.* **2011**, *50*, 1630–1634.
(5) Danishefsky, S.; Kitahara, T. *J. Am. Chem. Soc.* **1974**, *96*, 7807–7808.
(6) (a) Danishefsky, S. *Acc. Chem. Res.* **1981**, *14*, 400–406. (b) Herczegh, P.; Kovacs, I.; Erdoesi, G.; Varga, T.; Agocs, A.; Szilagyi, L.; Sztaricskai, F.; Berecibar, A.; Lukacs, G.; Olesker, A. *Pure Appl. Chem.* **1997**, *69*, 519–524. (c) Han, G.; LaPorte, M. G.; Folmer, J. J.; Werner, K. M.; Weinreb, S. M. *J. Org. Chem.* **2000**, *65*, 6293–6306.
(7) (a) Yu, Z.; Liu, X.; Dong, Z.; Xie, M.; Feng, X. *Angew. Chem., Int. Ed.* **2008**, *47*, 1308–1311. (b) Kozmin, S. A.; Rawal, V. H. *J. Org. Chem.* **1997**, *62*, 5252–5253. (c) Amii, H.; Kobayashi, T.; Terasawa, H.; Uneyama, K. *Org. Lett.* **2001**, *3*, 3103–3105.

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precedence for Danishefsky-type dienes that bear electron-withdrawing groups. We now report the synthesis of such a diene, compound **5**, as well as studies on its reactivity and use toward the synthesis of ansamycin antibiotics.

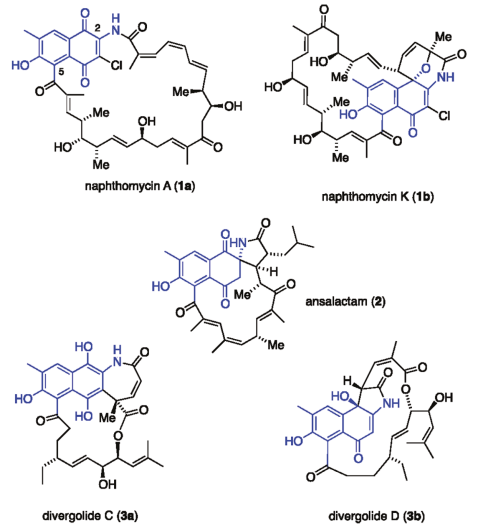
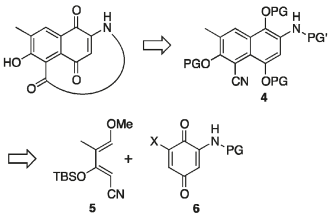


Figure 1. Structurally intriguing ansamycin antibiotics containing an aminonaphthoquinone core.

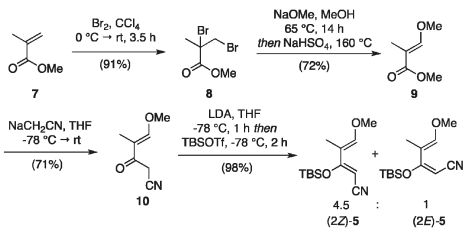
Scheme 1. Retrosynthetic Analysis of the Aminonaphthoquinone Core of Ansamycins with Suitable Functionalization



Our synthesis of **5** commenced with the bromination of commercially available methyl methacrylate[8] (**7**), followed by nucleophilic substitution and elimination to introduce a β-methoxy substituent (Scheme 2). Subsequent Claisen-type condensation with deprotonated acetonitrile gave ketonitrile **10**, which proved to be surprisingly stable. Next, conditions for its enolization and subsequent silylation were screened. Attempts to synthesize the TMS enol ether

(8) Werle, S.; Fey, T.; Neudoerfl, J. M.; Schmalz, H.-G. *Org. Lett.* **2007**, *9*, 3555–3558.

Scheme 2. Synthesis of Diene **5**



of **10** failed due to its high lability toward various workup conditions. However, we found that deprotonation with LDA and subsequent silylation with TBSOTf delivered silyloxy diene **5** as a 4.5:1 mixture of (2*Z*)- and (2*E*)-isomers in excellent overall yield. These isomers could be separated (see Supporting Information) but were usually employed as a mixture in subsequent reactions.

With multigram quantities of diene **5** in hand, we investigated its utility in the synthesis of naphthoquinones. Diels–Alder reaction of **5** with the known benzoquinone derivative **11**[9] gave intermediary product **12** as a mixture of stereoisomers, which was not further characterized. Treatment of this crude material with oven-dried silica gel in

Scheme 3. Synthesis of the Aminonaphthoquinone Core and Subsequent Reduction and Protection

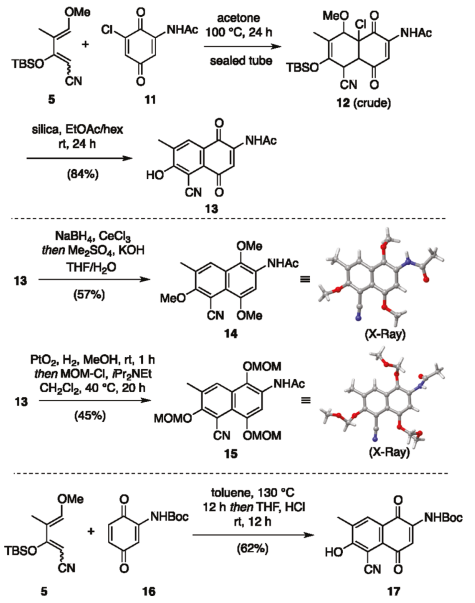
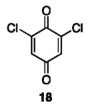
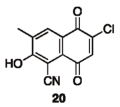
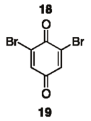
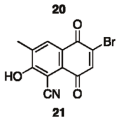
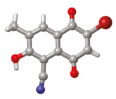
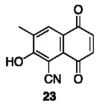
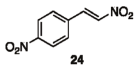
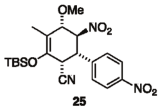
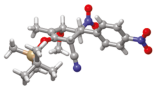
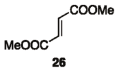
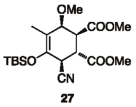
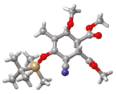
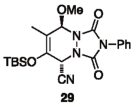
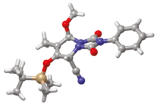


Table 1. Results of Diels–Alder Reactions of Diene **5** (4.5:1 Mixture of Stereoisomers) with Different Dienophiles

entry	dienophile	conditions	isolated product	crystal structure	yield [%]
1	 **18**	toluene, 120 °C, 29 h *then* silica, acetone, rt, 12 h	 **20**	–	79
2	 **19**	toluene, 150 °C, 3 h *then* THF, HCl, silica, rt, 12 h	 **21**		43
3	 **22**	$AlCl_3$, CH_2Cl_2, 0 °C, 3 h *then* silica	 **23**	–	79
4	 **24**	toluene, 120 °C, 2 h	 **25**		75
5	 **26**	toluene, 140 °C, 14 d	 **27**		23
6	 **28**	benzene, 80 °C, 12 h	 **29**		63

ethyl acetate/hexanes resulted in desilylation and aromatization to afford our key naphthoquionone **13** in 84% overall yield. Notably, only a single regioisomer was isolated.

To elaborate **13** into a more useful building block and confirm its structure, the naphthoquinone was reduced to the corresponding naphthohydroquinone using sodium borohydride. *In situ* protection of the phenolic hydroxy groups as methyl ethers then afforded hexasubstituted naphthalene **14**, the crystal structure of which is depicted in Scheme 3. Analogous protection of the three hydroxy groups as MOM ethers required reduction with hydrogen in the presence of Adam's catalyst, followed by treatment with MOM chloride and Hünig's base. This gave naphthalene derivative **15**, which was also characterized by X-ray crystallography. It should be noted that the alkylations required careful optimization to avoid *N*-methylation while ensuring that all three hydroxy groups were affected. Interestingly, the aromatization following the cycloaddition did not require an "inbuilt oxidant" in the form of a halogen substituent on the benzoquinone. Reaction of diene **5** with Moody's Boc-protected aminobenzoquinone **16**[10] gave naphthoquinone **17**, presumably through air oxidation of the intermediary cycloadduct.

To establish the synthetic scope of diene **5** in Diels–Alder reactions, we investigated its reactivity with other dienophiles (Table 1). Encouraged by our initial results, we first examined various quinones as dienophiles. Reaction of **5** with commercially available dichlorobenzoquinone (**18**) and the known dibromobenzoquinone (**19**)[11] provided naphthoquinones **20** and **21**, respectively, in satisfactory yields following aromatization (entries 1 and 2). When benzoquinone itself (**22**) was used as the dienophile, simple heating in toluene proved to be less effective than catalysis using $AlCl_3$ as a Lewis acid. Following aromatization, these catalytic conditions gave naphthoquinone **23** in good overall yield (entry 3). Reaction of **5** with nitrostyrene **24** afforded the desired cycloaddition product **25** as a single diastereomer (entry 4). Heating of diene **5** with dimethyl fumarate (**26**) over 14 days afforded cycloadduct **27** as a single diastereomer, but in only 23% yield. Reaction of **5** with phenyl triazoline dione (**28**) gave cycloadduct **29**,

(9) Kelly, T. R.; Echavarren, A.; Behforouz, M. *J. Org. Chem.* **1983**, *48*, 3849–3851.

(10) Nawrat, C. C.; Lewis, W.; Moody, C. J. *J. Org. Chem.* **2011**, *76*, 7872–7881.

(11) Omura, K. *Synthesis* **1998**, *8*, 1145–1148.

which has the opposite relative stereochemistry with respect to the OMe and CN substituents compared to **25** and **27**. This stereochemical outcome presumably reflects isomerization of the initial cycloadduct to the thermodynamically more stable product *via* reversible cleavage of the *N*,*O*-acetal. The structures of **21**, **25**, **27**, and **29** were confirmed by X-ray crystallography (Table 1 and Supporting Information). Attempted reactions of diene **5** with tetracyanoethylene or maleic anhydride failed to give the desired products despite extensive screening of conditions.

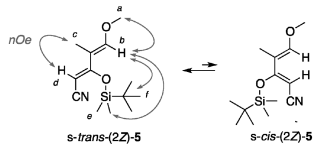


Figure 2. Conformational analysis of diene **5**.

From these data, it is apparent that diene **5** exhibits markedly reduced reactivity when compared to the parent Danishefsky diene. This can be partially attributed to the electronic effect of the cyano substituent but is probably also due to the influence of the methyl substituent on the preferred conformation of the diene. To undergo a [4 + 2] cycloaddition, **5** must adopt an s-*cis* conformation (Figure 2). NMR spectroscopy of pure (2*Z*)-**5** demonstrated a strong NOE correlation between olefinic proton *d* and the protons of the methyl group along with weak interactions between olefinic proton *b* and protons *e* and *f* of the TBS group. By contrast, no NOE could be observed between protons *b* and *d* or between proton *c* and the protecting group substituents. This strongly suggests that (2*Z*)-**5** mostly adopts an s-*trans* conformation and that the requisite s-*cis* conformation is sparsely populated. We assume that this effect is even more pronounced in the (2*E*)-isomer of **5** and that this isomer is essentially unreactive in Diels–Alder cycloadditions, or it may isomerize to its (2*Z*)-diastereomer under the reaction conditions.

In summary, we have reported the synthesis of a novel Danishefsky-type diene, which allows for the rapid assembly of substituted aminonaphthoquinones or other highly functionalized small molecules. Related studies on Diels–Alder dienes that bear substituents with opposing electronic effects will be further pursued. Our ongoing attempts to implement our synthetic strategy in the total synthesis of ansamycin antibiotics will also be reported in due course.

Acknowledgment. We thank Dr. Rob Webster (Ludwig-Maximilians-Universität München) for helpful discussions.

Supporting Information Available. Experimental procedures, spectroscopic and analytical data for compounds **5**–**29**, and X-ray data for compounds **14**, **15**, **21**, **25**, **27**, and **29**. This material is available free of charge via the Internet at http://pubs.acs.org.

The authors declare no competing financial interest.

An approach to aminonaphthoquinone ansamycins using a modified Danishefsky diene

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Supplementary Information

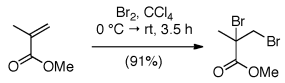
Index:

General Experimental Details. Unless stated otherwise, all reactions were performed in oven-dried or flame-dried glassware under a positive pressure of nitrogen. Commercial reagents and solvents were used as received with the following exceptions. Tetrahydrofuran (THF) was distilled from benzophenone and sodium immediately prior to use. Triethylamine, diisopropylamine and diisopropylethylamine were distilled over calcium hydride immediately before use. Reactions were magnetically stirred and monitored by NMR spectroscopy or analytical thin-layer chromatography (TLC) using E. Merck 0.25 mm silica gel 60 F_{254} precoated glass plates. TLC plates were visualized by exposure to ultraviolet light (UV, 254 nm) and/or exposure to an aqueous solution of ceric ammoniummolybdate (CAM), an aqueous solution of potassium permanganate ($KMnO_4$), an acidic solution of vanillin or a solution of ninhydrin in ethanol followed by heating with a heat gun. Flash column chromatography was performed as described by Still *et al.* employing silica gel (60 Å, 40-63 μm, Merck) and a forced flow of eluant at 1.3–1.5 bar pressure.[1] Yields refer to chromatographically and spectroscopically (^{1}H and ^{13}C NMR) pure material.

Instrumentation. Proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded on Varian VNMRS 300, VNMRS 400, INOVA 400 or VNMRS 600 spectrometers. Proton chemical shifts are expressed in parts per million (δ scale) and are calibrated using residual undeuterated solvent as an internal reference ($CHCl_3$: δ 7.26, DMSO-d_6: δ 2.50, $(CD_3)_2CO$: δ 2.05, CD_3OD: δ 3.31). Data for ^{1}H NMR spectra are reported as follows: chemical shift (δ ppm) (multiplicity, coupling constant (Hz), integration). Multiplicities are reported as follows: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, *br* = broad, app = apparent, or combinations thereof. Carbon nuclear magnetic resonance (^{13}C NMR) spectra were recorded on Varian VNMRS 300, VNMRS 400, INOVA 400 or VNMRS 600 spectrometers. Carbon chemical shifts are expressed in parts per million (δ scale) and are referenced to the carbon resonances of the solvent ($CDCl_3$: δ 77.0, DMSO-d_6: δ 39.5, $(CD_3)_2CO$: δ 29.8, CD_3OD: δ 49.00). Infrared (IR) spectra were recorded on a Perkin Elmer Spectrum BX II (FTIR System). IR data is reported in frequency of absorption (cm^{-1}). Mass spectroscopy (MS) experiments were performed on a Thermo Finnigan MAT 95 (EI) or on a Thermo Finnigan LTQ FT (ESI) instrument.

[1] Still, W. C.; Kahn, M.; Mitra, A. *J. Org. Chem.* **1978**, *43*, 2923–2925.

Synthetic procedures.



7 8

Methyl 2,3-dibromo-2-methylpropanoate (8):

Br_2 (5.27 mL, 103 mmol, 1.03 equiv) in CCl_4 (40 mL) was added dropwise over 2.5 h to a solution of methyl methacrylate (**7**) (10.7 mL, 99.9 mmol, 1.00 equiv) in CCl_4 (100 mL) at 0 °C. After complete addition, the orange solution was stirred at this temperature for further 1 h. A solution of sat. aq. $Na_2S_2O_3$ (70 mL) was then added to the colorless reaction mixture at 0 °C and it was allowed to warm to rt. The solution was extracted with TBME (1 × 200 mL then 3 × 100 mL) and the combined organic phases were dried over Na_2SO_4 and the solvent evaporated to afford dibromide **8** (23.5 g, 90.4 mmol, 91%) as a pale yellow liquid.

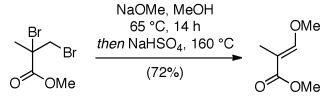
TLC (hexanes:EtOAc = 5:1), R_f = 0.86 (UV/CAM)

^{1}H NMR (300 MHz, $CDCl_3$) δ: 4.22 (dd, *J* = 9.8 Hz, 0.7 Hz, 1 H), 3.83 (s, 3 H), 3.72 (d, *J* = 9.8 Hz, 1 H), 2.03 (d, *J* = 0.7 Hz, 3 H).

^{13}C NMR (75 MHz, $CDCl_3$) δ: 169.1, 55.3, 53.4, 38.1, 26.4.

IR (Diamond-ATR, neat) ν_{max}: 1741, 1450, 1381, 1291, 1235, 1197, 1169, 1103, 1078, 1048, 990, 925, 871, 831, 772, 666 cm^{-1}.

HRMS (EI) calcd for $C_5H_8{}^{79}Br^{81}BrO_2$ $[M]^{+\bullet}$: 259.8871; found: 259.9019.



8 9

(*E*)-methyl 3-methoxy-2-methylacrylate (9):

Freshly cut sodium (7.77 g, 338 mmol, 2.00 equiv) was dissolved in methanol (120 mL) and the highly viscous solution heated to 68 °C. A solution of **8** (43.98 g, 169 mmol, 1.00 equiv) in MeOH (50 mL) was added rapidly and the mixture stirred at 68 °C for 14 h. The reaction mixture was allowed to cool to rt and filtered. The filter residue was washed with a small amount of cold MeOH, the filtrate concentrated to 1/3 of its volume *in vacuo* and filtered again. To the resulting solution was added H_2O (30 mL) and the biphasic system was extracted with Et_2O (4 × 60 mL). The combined organic layers were dried over Na_2SO_4 and

the solvent was evaporated. $NaHSO_4 \cdot H_2O$ (160 mg, 1.16 mmol, 0.006 equiv) was added to the reaction mixture and the suspension was heated to 160 °C under ambient pressure. When no more evolution of MeOH was observed, the residue was subjected to fractional distillation (90 °C, 45 mbar) to afford the product **9** (15.8 g, 71.8 mmol, 72%) as a colorless oil.

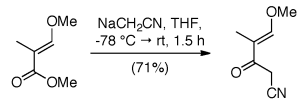
TLC (hexanes:EtOAc = 2:1), R_f = 0.63 (UV/CAM)

^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.27–7.26 (m, 1 H), 3.94 (s, 3 H), 3.62 (s, 3 H), 1.75 (d, *J* = 1.3 Hz, 3 H).

^{13}C-NMR (75 MHz, $CDCl_3$) δ = 169.2, 158.5, 106.1, 61.1, 51.2, 9.0.

IR (Diamond-ATR, neat) ν_{max}: 2950, 1706, 1645, 1436, 1389, 1356, 1295, 1241, 1189, 1144, 1112, 1024, 993, 943, 905, 836, 757, 718 cm^{-1}.

HRMS (EI) calcd for $C_6H_{10}O_3$ $[M]^{+\bullet}$: 130.0630; found: 130.0621.



9 **10**

(*E*)-5-methoxy-4-methyl-3-oxopent-4-enenitrile (10):

To a solution of NaHMDS (1 M solution in THF, 7.74 mL, 7.74 mmol, 2.2 equiv) in THF (10 mL) was added MeCN (0.441 mL, 8.45 mmol, 2.4 equiv) dropwise at -78 °C. After 20 minutes, this solution was transferred via canula to a solution of **9** (458 mg, 3.52 mmol, 1.0 equiv) in THF (40 mL) at -78 °C over a period of 20 minutes. The reaction mixture was maintained at -78 °C for 30 minutes and then warmed to 0 °C. After 40 minutes, the reaction was quenched by addition of sat. aq. NH_4Cl (30 mL) and subsequently extracted with Et_2O (3 × 50 mL) and EtOAc (2 × 50 mL). The combined organic layers were dried over Na_2SO_4, filtered and concentrated under reduced pressure. The crude orange oil, which tended to crush out in less polar solvents, was dissolved in a small amount of $CHCl_3$ and purified by flash column chromatography (silica gel packed in $CHCl_3$, gradient: hexanes:EtOAc = 2:1 → 1:1) to afford the title compound **10** (350 mg, 2.52 mmol, 71%) as a white solid.

TLC (hexanes:EtOAc = 1:1), R_f = 0.36 (UV/$KMnO_4$)

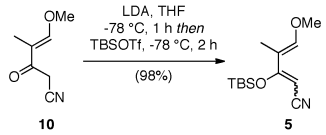
M.p.: 101–103 °C

^{1}H NMR (600 MHz, $CDCl_3$) δ: 7.26 (dd, *J* = 2.4, 1.2 Hz, 1 H), 3.94 (s, 3 H), 3.62 (s, 2 H), 1.75 (d, *J* = 1.2 Hz, 3 H).

^{13}C NMR (150 MHz, $CDCl_3$) δ: 186.0, 161.7, 115.8, 114.5, 62.1, 28.1, 8.5.

IR (Diamond-ATR, neat) ν_{max}: 2956, 2920, 2259, 1652, 1626, 1452, 1410, 1393, 1369, 1336, 1256, 1217, 1149, 1059, 993, 967, 912, 890, 824, 713 cm^{-1}.

HRMS (EI) calcd for $C_7H_9NO_2$ $[M]^{+\bullet}$: 139.0633; found: 139.0635.



10 **5**

(2*Z*,4*E*)-3-((tert-butyldimethylsilyl)oxy)-5-methoxy-4-methylpenta-2,4-dienenitrile (5):

To a solution of diisopropylamine (2.54 mL, 18.0 mmol, 1.25 equiv) in THF (60 mL) was added *n*-BuLi (2.5 M solution in hexanes, 6.3 mL, 15.8 mmol, 1.10 equiv) dropwise at -78 °C. The solution was stirred at -78 °C for 10 min, warmed to 0 °C and stirred at this temperature for 15 min and subsequently cooled back to -78 °C. A solution of **10** in THF (20 mL) was then added to the freshly prepared LDA solution. After stirring at -78 °C for 2 h, TBSOTf was added to the orange reaction mixture and the solution was stirred for 1 h. A 1:1 mixture of H_2O (20 mL) and sat. aq. NH_4Cl (20 mL) and EtOAc (30 mL) were added and the reaction mixture was warmed to rt. The organic phase was separated and the aq. phase was extracted with EtOAc (3 × 50 mL). The combined organic layers were dried over Na_2SO_4, filtered and concentrated under reduced pressure. The resulting yellow oil was purified by flash column chromatography (silica gel, hexanes:EtOAc = 20:1) to provide silyl-enol ether **5** (3.57 g, 14.1 mmol, 98%) as a pale-yellow oil.

Note: Since the product hydrolyzes on silica gel, it is recommended to quickly flush the crude over a relatively short column.

TLC (hexanes:EtOAc = 20:1), R_f = 0.46 (2*Z*)-**5**, 0.38 (2*E*)-**5** (UV/ $KMnO_4$)

(2*E*)-**5**:

^{1}H NMR (400 MHz, $CDCl_3$) δ: 6.90 (q, *J* = 1.3 Hz, 1 H), 4.38 (s, 1 H), 3.77 (s, 3 H), 1.87 (d, *J* = 1.2 Hz, 3 H), 0.95 (s, 9 H), 0.22 (s, 6 H).

^{13}C NMR (150 MHz, $CDCl_3$) δ: 170.5, 153.5, 119.2, 110.4, 74.0, 60.8, 25.5, 18.2, 10.7, -4.6.

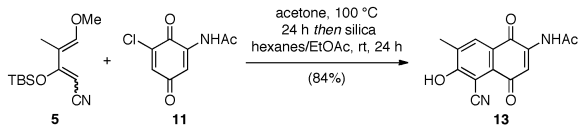
(2*Z*)-**5**:

^{1}H NMR (300 MHz, $CDCl_3$) δ: 6.75 (q, *J* = 1.2 Hz, 1 H), 4.55 (s, 1 H), 3.76 (s, 3 H), 1.67 (d, *J* = 1.1 Hz, 3 H), 1.03 (d, *J* = 0.2 Hz, 9 H), 0.28 (d, *J* = 0.3 Hz, 6 H).

^{13}C NMR (75 MHz, $CDCl_3$) δ: 168.6, 152.5, 118.5, 110.2, 74.6, 60.9, 25.8, 18.5, 9.9, -3.6.

IR (Diamond-ATR, neat) ν_{max}: 2954, 2932, 2887, 2860, 2208, 1704, 1641, 1585, 1472, 1464, 1394, 1368, 1329, 1238, 1141, 1116, 1046, 1004, 978, 939, 893, 841, 824, 807, 783, 746, 702, 679, 637 cm^{-1}.

HRMS (EI) calcd for $C_{13}H_{23}NO_2Si$ $[M]^{+\bullet}$: 253.1498; found: 253.1502.



Aminonaphthoquinone 13:

Quinone **11**[2] (501 mg, 2.51 mmol, 1.0 equiv) and a solution of diene **5** (700 mg, 2.76 mmol, 1.1 equiv) in acetone (10 mL) were combined in a pressure tube and heated to 100 °C for 24 h under an atmosphere of argon. After evaporation of the solvent, the brown residue was suspended in a 1:1 mixture of hexanes/EtOAc (50 mL), oven-dried silica (5 g) was added and the mixture was stirred overnight. The solvent was removed and the residue was purified by flash column chromatography (silica gel, hexanes:EtOAc = 1:1 → $CHCl_3$/acetone = 5:1 → 1:2) to provide aminonaphthoquinone **13** (624 mg, 2.31 mmol, 84%) as a purple-black solid.

M.p.: decomposition without melting

TLC (CH_2Cl_2:acetone:AcOH:H_2O = 70:10:0.5:0.5), R_f = 0.45 (visible/CAM)

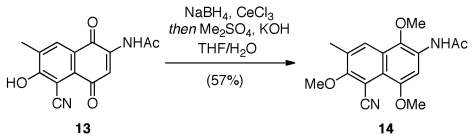
^{1}H NMR (300 MHz, DMSO-d_6) δ: 9.56 (s, 1 H), 7.58 (d, J = 0.9 Hz, 1 H), 7.28 (s, 1 H), 2.20 (s, 3 H), 1.97 (d, J = 0.9 Hz, 3 H).

^{13}C NMR (75 MHz, DMSO-d_6) δ: 186.1, 177.7, 175.4, 170.9, 141.0, 135.1, 133.4, 128.7, 119.2, 113.3, 111.2, 94.7, 24.5, 17.1.

IR (Diamond-ATR, neat) ν_{max}: 3302, 2213, 1648, 1581, 1490, 1365, 1335, 1273, 1212, 1086, 1013, 874, 852, 805, 742, 706 cm^{-1}.

HRMS (ESI) calcd for $C_{14}H_9N_2O_4$ $[M\text{-}H]^-$: 269.0568; found: 269.0567.

[2] Kelly, T. R.; Echavarren, A.; Behforouz, B. *J. Org. Chem.* **1983**, *48*, 3849–3851.



Cyano naphthalene 14:

A solution of **13** (80 mg, 0.29 mmol, 1.00 equiv) in a mixture of THF (10 mL) and H_2O (5 mL) was degassed with Argon in a sonicator for 5 minutes and Cerium(III) chloride heptahydrate (162 mg, 0.44 mmol, 1.50 equiv) was added. The reaction mixture was cooled to 0 °C and $NaBH_4$ (20 mg, 0.52 mmol, 1.80 equiv) was added in two portions over 20 min. After H_2 evolution had ceased, the reaction was warmed to rt, Me_2SO_4 (550 µL, 5.80 mmol, 20 equiv) was added and the flask was evacuated and filled with argon. A solution of KOH (325 mg, 5.80 mmol, 20 equiv) in H_2O (5 mL) was added dropwise. The reaction mixture was stirred at rt for 15 h, cooled to 0 °C and conc. NH_4OH (3 mL) and H_2O (10 mL) were added. The reaction mixture was extracted with EtOAc (6 × 30 mL) and the combined organic layers were dried over Na_2SO_4, filtered and concentrated under reduced pressure. Purification of the residue by flash column chromatography (silica gel, gradient: $CHCl_3$:acetone = 20:1 → 10:1) afforded the title compound **14** (52 mg, 0.17 mmol, 57%) as an orange solid.

TLC ($CHCl_3$:acetone = 3:1), R_f = 0.67 (UV/CAM)

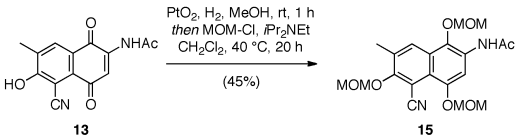
M.p.: 215–220 °C

^{1}H NMR (600 MHz, $CDCl_3$) δ: 8.09 (s, 1 H), 7.93 (s, 1 H), 7.78 (*br* s, 1 H), 4.05 (s, 3 H), 4.03 (s, 3 H), 2.47 (d, *J* = 0.8 Hz, 3 H), 2.28 (s, 3 H).

^{13}C NMR (150 MHz, $CDCl_3$) δ: 168.5, 163.1, 151.1, 136.1, 132.2, 128.5, 127.6, 124.9, 120.6, 116.5, 101.1, 99.7, 61.8, 61.7, 56.0, 25.0, 16.9.

IR (Diamond-ATR, neat) ν_{max}: 3241, 2941, 2221, 1693, 1659, 1622, 1602, 1497, 1458, 1443, 1400, 1364, 1350, 1278, 1234, 1213, 1168, 1144, 1097, 1055, 1001, 968, 894, 865, 848, 818, 789, 732, 707, 683 cm^{-1}.

HRMS (ESI) calcd for $C_{17}H_{19}N_2O_4$ $[M+H]^+$: 315.1345; found: 315.1336.



Naphthalene 15:

To a suspension of **13** (100 mg, 0.37 mmol, 1.00 equiv) in MeOH (6 mL) was added PtO_2 (12.6 mg, 55.5 µmol, 0.15 equiv) and the inner atmosphere of the flask was exchanged three times with hydrogen and the resulting mixture was stirred under hydrogen atmosphere (double layer balloon) for 1 h at rt. The reaction mixture was filtered through a syringe filter and the solvent was removed under reduced pressure under exclusion of air. The residue was dissolved in CH_2Cl_2 (10 mL), Chloromethyl methyl ether (298 mg, 3.70 mmol, 10 equiv) and diisopropylamine (0.83 mL, 4.81 mmol, 13 equiv) were added and the reaction mixture was sealed with a yellow cap and stirred at 40 °C. After 20 h, the reaction mixture was cooled to rt and the solvent was removed *in vacuo*. Purification of the residue by flash column chromatography (silica gel, gradient: $CHCl_3$:acetone = 10:1 → 2:1) afforded the title compound **15** (68 mg, 168 mµol, 45%) as a white solid.

TLC ($CHCl_3$:acetone = 10:1), R_f = 0.27 (UV/CAM)

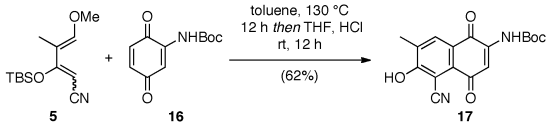
M.p.: 120–122 °C

^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.58 (*br* s, 1 H), 8.31 (s, 1 H), 7.92 (d, *J* = 0.7 Hz, 1 H), 5.40 (s, 2 H), 5.32 (s, 2 H), 5.10 (s, 2 H), 3.70 (s, 3 H), 3.66 (s, 3 H), 3.61 (s, 3 H), 2.49 (d, *J* = 0.9 Hz, 3 H), 2.22 (s, 3 H).

^{13}C NMR (100 MHz, $CDCl_3$) δ: 168.4, 161.3, 148.4, 135.8, 132.4, 129.1, 127.7, 125.8, 120.9, 116.8, 105.4, 101.2, 100.9, 100.0, 95.1, 58.2, 57.7, 56.9, 24.8, 17.6.

IR (Diamond-ATR, neat) ν_{max}: 3252, 2922, 2829, 2361, 2338, 1662, 1620, 1605, 1526, 1497, 1430, 1398, 1413, 1360, 1347, 1284, 1242, 1152, 1144, 1086, 1078, 1035, 986, 967, 919, 888, 820, 734, 668 cm^{-1}.

HRMS (EI) calcd for $C_{20}H_{24}N_2O_7Na$ $[M+Na]^+$: 427.1476; found: 427.1477.



***N*-Boc naphthoquinone 17:**

To a solution of **5** (37 mg, 0.15 mmol, 1.00 equiv) in toluene (3.5 mL) in a pressure tube was added **16**[3] (33 mg, 0.15 mmol, 1.00 equiv). The reaction mixture was stirred for 12 h at 130 °C and subsequently cooled to rt. The solvent was evaporated, the crude product was dissolved in THF (5 mL) and aq. HCl (2 M, 3 drops) was added and the reaction mixture was stirred for further 12 h at rt. Silica (2 g) was added to the solution and the solvent removed *in vacuo*. Purification by flash column chromatography (silica gel, gradient: $CHCl_3$:acetone = 1:1 → 1:9) yielded the desired product **17** (30 mg, 0.091 mmol, 62%) as a purple solid.

TLC ($CHCl_3$:acetone = 1:2), R_f = 0.31 (visible/$KMnO_4$)

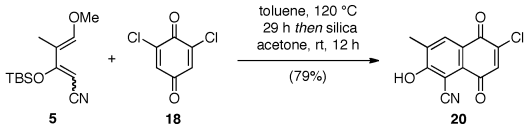
M.p.: decomposition without melting

^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.20 (s, 1 H), 7.56 (d, J = 1.0 Hz, 1 H), 6.84 (s, 1 H), 1.96 (d, J = 0.9 Hz, 3 H), 1.48 (s, 9 H).

^{13}C NMR (100 MHz, DMSO-d_6) δ: 185.2, 177.9, 174.5, 151.2, 141.3, 135.5, 133.2, 128.8, 119.2, 111.0, 95.3, 90.6, 81.5, 27.7, 17.1.

IR (Diamond-ATR, neat) ν_{max}: 3361, 2223, 1732, 1650, 1584, 1503, 1476, 1367, 1333, 1272, 1147, 1032, 844 cm^{-1}.

HRMS (ESI) calcd for $C_{17}H_{16}N_2O_5$ $[M+Na]^+$: 351.0957; found: 351.0953.



Chloro naphthoquinone 20:

A solution of diene **5** (424 mg, 1.67 mmol, 1.50 equiv) in toluene (8 mL) was added to 2,6-dichlorobenzoquinone (**18**) (187 mg, 1.12 mmol, 1.00 equiv) in a pressure tube. The brown-orange mixture was heated to 120 °C for 29 h. The solvent was removed *in vacuo* and the brown, amorphous residue dissolved in acetone (30 mL). Silica gel (5 g) was added and the brown-purple slurry stirred at ambient temperature for 12 h. The solvent was evaporated and

[3] Nawrat, C. C.; Lewis, W.; Moody, C. J. *J. Org. Chem.* **2011**, *76*, 7872–7881.

the residue was purified by flash column chromatography (silica gel, $CHCl_3$:acetone = 1:2) to yield naphthoquinone **20** (220 mg, 0.89 mmol, 79%) as a dark purple solid.

TLC ($CHCl_3$:acetone = 1:10), R_f = 0.19 (visible/CAM)

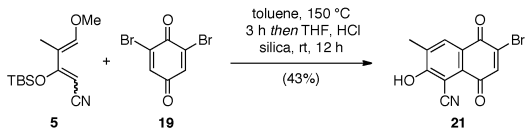
M.p.: no melting point or visible decomposition in the range of 20-400 °C

^{1}H NMR (400 MHz, DMSO-d_6) δ: 7.60 (d, J = 1.0 Hz, 1 H), 7.07 (s, 1 H), 1.99 (d, J = 1.0 Hz, 3 H).

^{13}C NMR (100 MHz, DMSO-d_6) δ: 183.3, 176.8, 173.4, 145.4, 134.5, 134.5, 133.5, 129.3, 118.9, 112.9, 94.8, 17.2.

IR (Diamond-ATR, neat) ν_{max}: 3571, 3358, 3052, 2218, 1675, 1650, 1599, 1577, 1534, 1463, 1421, 1355, 1324, 1258, 1210, 1036, 1006, 936, 924, 905, 892, 826, 800, 717, 654 cm^{-1}.

HRMS (EI) calcd for $C_{12}H_5{}^{35}ClNO_3$ $[M]^{+\bullet}$: 245.9958; found: 245.9962.



Bromo naphthoquinone 17:

A solution of diene **5** (200 mg, 0.79 mmol, 1.50 equiv) in toluene (5 mL) was added to 2,6-dibromobenzoquinone (**19**)[4] (140 mg, 0.53 mmol, 1.00 equiv) in a pressure tube. The orange mixture was heated to 150 °C for 3 h. The solvent was removed *in vacuo* and the residue dissolved in THF (5 mL). Conc. HCl (3 drops) and silica gel (2 g) was added and the brown-purple slurry stirred at ambient temperature for 12 h. The solvent was evaporated and the residue was purified by flash column chromatography (silica gel, gradient: $CHCl_3$:acetone = 1:1 → 1:3) gave naphthoquinone **21** (66 mg, 0.23 mmol, 43%) as a dark purple solid.

TLC (CH_2Cl_2:acetone:AcOH:H_2O = 70:10:0.5:0.5), R_f = 0.5 (visible/CAM)

M.p.: decomposition without melting

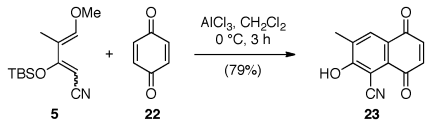
^{1}H NMR (600 MHz, DMSO-d_6) δ: 8.03 (d, J = 0.9 Hz, 1 H), 7.67 (s, 1 H), 2.35 (d, J = 0.9 Hz, 3 H).

^{13}C NMR (150 MHz, DMSO-d_6) δ: 180.7, 175.9, 140.0, 138.3, 133.1, 132.6, 132.6, 122.9, 114.8, 96.8, 90.6, 16.9.

[4] Omura, K. *Synthesis* **1998**, *8*, 1145–1148.

IR (Diamond-ATR, neat) ν_{max}: 2361, 2340, 1662, 1576, 1539, 1492, 1473, 1456, 1436, 1367, 1321, 1252, 1054, 1032, 1004, 911, 884, 826, 813, 798, 699 cm^{-1}.

HRMS (ESI) calcd for $C_{12}H_5BrNO_3$ $[M-H]^-$: 289.9458; found: 289.9462.



Naphthoquinone 23:

$AlCl_3$ (2.1 mg, 15.7 µmol, 0.11 equiv) was added to a solution of diene **5** (35.4 mg, 140 µmol, 1.00 equiv) and *p*-benzoquinone (**22**) (30.2 mg, 279 µmol, 2.00 equiv) in CH_2Cl_2 (0.7 mL) at 0 °C. The mixture was stirred at this temperature for 3 h and turned dark green. H_2O (1 mL) was added and the mixture was concentrated *in vacuo*. The yellow residue was purified by flash column chromatography (silica gel, $CHCl_3$:acetone = 1:4) to afford the desired product **23** (23.6 mg, 111 µmol, 79%) as a dark purple solid.

TLC ($CHCl_3$:acetone = 1:2), R_f = 0.14 (visible/CAM)

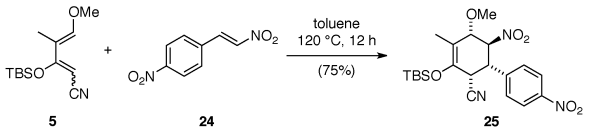
M.p.: No melting point or visible decomposition in the range of 20-400 °C.

^{1}H NMR (300 MHz, DMSO-d_6) δ: 7.55 (d, J = 1.0 Hz, 1 H), 6.70 (s, 2 H), 2.01 (d, J = 1.0 Hz, 3 H).

^{13}C NMR (75 MHz, DMSO-d_6) δ: 185.6, 181.4, 176.4, 138.6, 136.4, 134.5, 134.1, 128.6, 119.2, 114.4, 93.9, 17.3.

IR (Diamond-ATR, neat) ν_{max}: 3347, 2921, 2212, 1668, 1651, 1582, 1539, 1500, 1456, 1389, 1374, 1361, 1327, 1273, 1192, 1164, 1084, 1026, 986, 845, 810, 765, 687, 625 cm^{-1}.

HRMS (ESI) calcd for $C_{12}H_6NO_3$ $[M-H]^-$: 212.0353; found: 212.0352.



Cycloadduct 25:

To a solution of **5** (30 mg, 118 µmol, 1.00 equiv) in toluene (2 mL) was added **24** (25 mg, 129 µmol, 1.09 equiv) in a pressure tube and the reaction mixture was heated to 120 °C for

12 h. The solvent was removed *in vacuo* and the residue was purified by flash column chromatography (silica gel, gradient: hexanes:EtOAc = 15:1 → 10:1 → 5:1) to afford the desired product **25** (39 mg, 87.1 µmol, 75%) as a colorless wax.

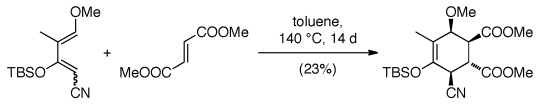
TLC (hexanes:EtOAc = 5:1), R_f = 0.47 (UV/CAM)

^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.29–8.25 (m, 1 H), 7.60–7.56 (m, 1 H), 5.47–5.40 (dd, *J* = 12.5, 8.1 Hz, 1 H), 4.66 (d, *J* = 8.1 Hz, 1 H), 3.87 (dd, *J* = 12.6, 4.9 Hz, 1 H), 3.41 (s, 3 H), 3.30 (d, *J* = 4.6 Hz, 1 H), 1.77 (s, 3 H), 0.97 (s, 9 H), 0.26 (d, *J* = 2.2 Hz, 6 H).

^{13}C NMR (75 MHz, $CDCl_3$) δ: 148.5, 140.4, 139.9, 129.1, 124.5, 116.3, 115.3, 85.8, 81.3, 55.6, 44.5, 39.8, 25.7, 18.3, 12.6. -3.7, -4.0.

IR (Diamond-ATR, neat) ν_{max}: 2933, 2898, 1684, 1640, 1602, 1556, 1518, 1470, 1412, 1378, 1343, 1293, 1260, 1222, 1194, 1184, 1111, 1069, 966, 951, 913, 838, 823, 808, 782, 774, 742 cm^{-1}.

HRMS (ESI) calcd for $C_{21}H_{29}N_3O_6Si$ $[M-H]^-$: 446.1826, found 446.1759.



Methylester 27:

A solution of diene **5** (88.9 mg, 0.35 mmol, 1.00 equiv) in toluene (3 mL) and dimethylfumarate (**26**) (75.8, 0.53 mmol, 1.50 equiv) were dissolved in toluene and the resulting mixture heated in a pressure tube to 140 °C fourteen days. The solvent was evaporated and the resulting crude brown solid purified by flash column chromatography (silica gel, hexanes:EtOAc = 8:1) to afford the title compound **27** (32.1 mg, 80.7 µmol, 23%) as a white amorphous solid.

TLC (hexanes:EtOAc = 8:1), R_f = 0.25 (UV/$KMnO_4$)

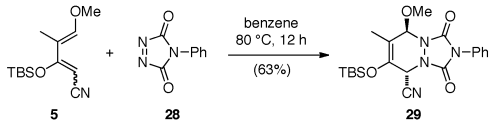
M.p.: 105 °C

^{1}H NMR (400 MHz, $CDCl_3$) δ: 4.06 (d, *J* = 3.4 Hz, 1 H), 3.83 (s, 3 H), 3.74 (s, 3 H), 3.50 (ddd, *J* = 12.2, 11.0, 0.3 Hz, 1 H), 3.39 (s, 3 H), 3.28 (ddq, *J* = 11.0, 2.1, 0.8 Hz, 1 H), 2.88 (dd, *J* = 12.2. Hz, 3.4 Hz, 1 H), 1.77 (dd, *J* = 2.1 Hz, 0.2 Hz, 1 H), 0.99 (s, 9 H), 0.19 (s, 3 H), 0.15 (s, 3 H).

^{13}C NMR (100 MHz, $CDCl_3$) δ: 173.3, 170.7, 138.0, 116.9, 115.6, 78.1, 60.0, 52.8, 52.2, 46.8, 41.5, 36.5, 25.6, 18.2, 15.7, -3.8, -4.2.

IR (Diamond-ATR, neat) ν_{max}: 2954, 2932, 2888, 2859, 1730, 1690, 1473, 1462, 1436, 1381, 1350, 1314, 1276, 1254, 1230, 1178, 1165, 1011, 978, 947, 912, 872, 841, 827, 815, 782, 747, 714, 687, 608 cm^{-1}.

HRMS (ESI) calcd for $C_{19}H_{31}NO_6NaSi$ $[M+Na]^+$: 420.1813, found 420.1812.



Cycloadduct 29:

To a solution of **5** (42 mg, 166 µmol, 1.00 equiv) in benzene (3 mL) was added **28** (32 mg, 182 µmol, 1.10 equiv) in a pressure tube and the reaction mixture was heated to 80 °C for 12 h. The solvent was removed *in vacuo* and the residue was purified by flash column chromatography (silica gel, gradient: hexanes:EtOAc = 10:1 → 5:1 → 2:1) to afford the desired product **29** (45 mg, 0.11 mmol, 63%) as a white crystalline solid.

TLC (hexanes:EtOAc = 5:1), R_f = 0.24 (UV/CAM)

M.p.: 102 °C

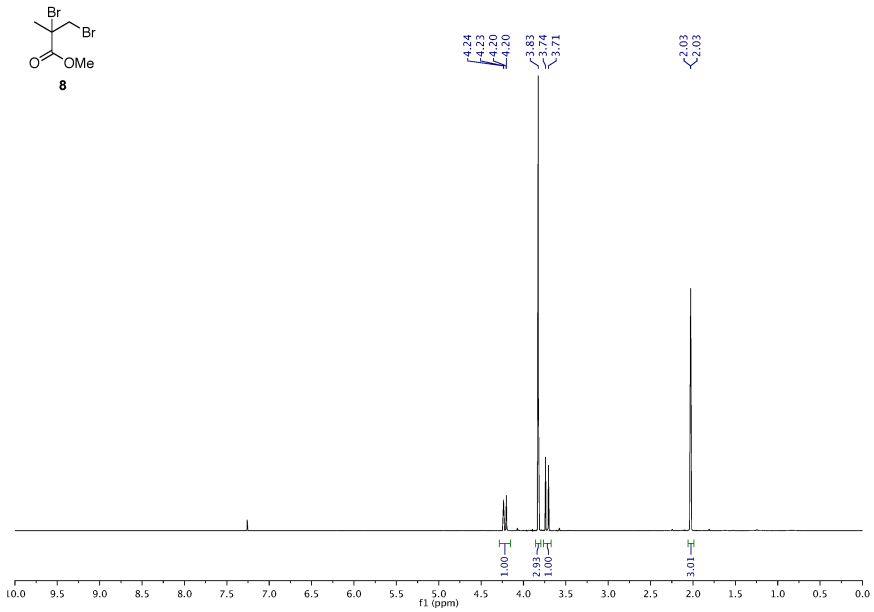
^{1}H NMR (600 MHz, $CDCl_3$) δ: 7.54–7.48 (m, 4 H), 7.44–7.38 (m, 1 H), 5.57 (s, 1 H), 4.98 (m, 1 H), 3.55 (s, 3 H), 1.83 (d, J = 1.4 Hz, 3 H), 1.02 (s, 9 H), 0.28 (d, J = 4.8 Hz, 6 H).

^{13}C NMR (150 MHz, $CDCl_3$) δ: 151.3, 150.4, 137.5, 130.5, 129.3, 129.3, 128.7, 125.3, 113.2, 113.1, 83.1, 56.1, 47.1, 25.5, 18.2, 13.3, -3.8, -3.9.

IR (Diamond-ATR, neat) ν_{max}: 2953, 2926, 2856, 1729, 1689, 1461, 1436, 1379, 1350, 1313, 1274, 1253, 1229, 1177, 1164, 1077, 1010, 978, 946, 911, 871, 840, 826, 814, 781, 746, 714, 686 cm^{-1}.

HRMS (ESI) calcd for $C_{21}H_{28}N_4O_4NaSi$ $[M+Na]^+$: 451.1772, found 451.1777.

NMR spectra.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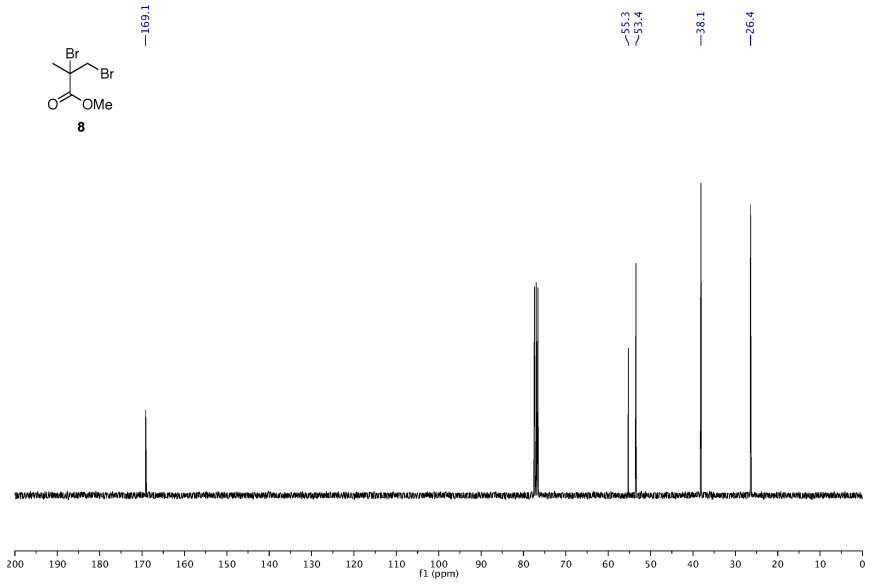
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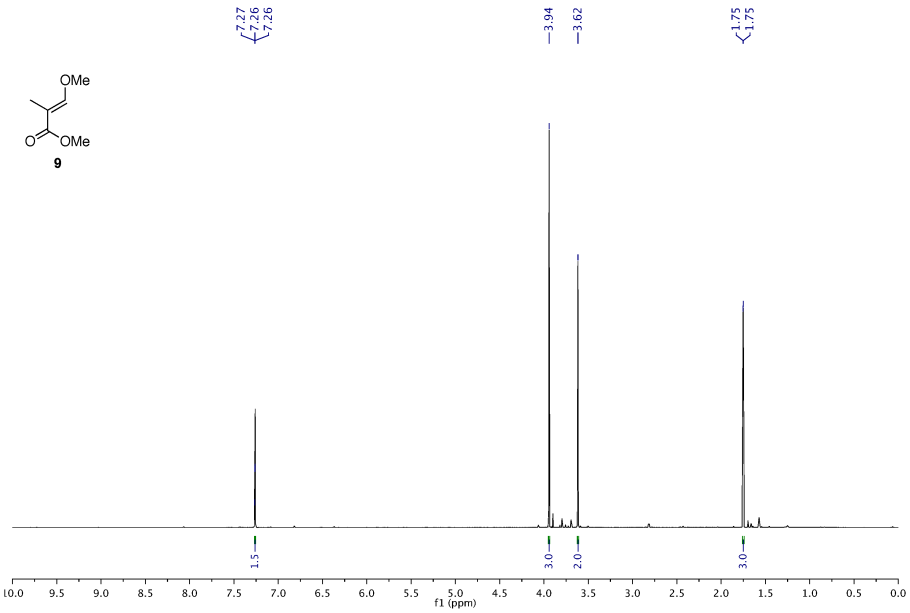
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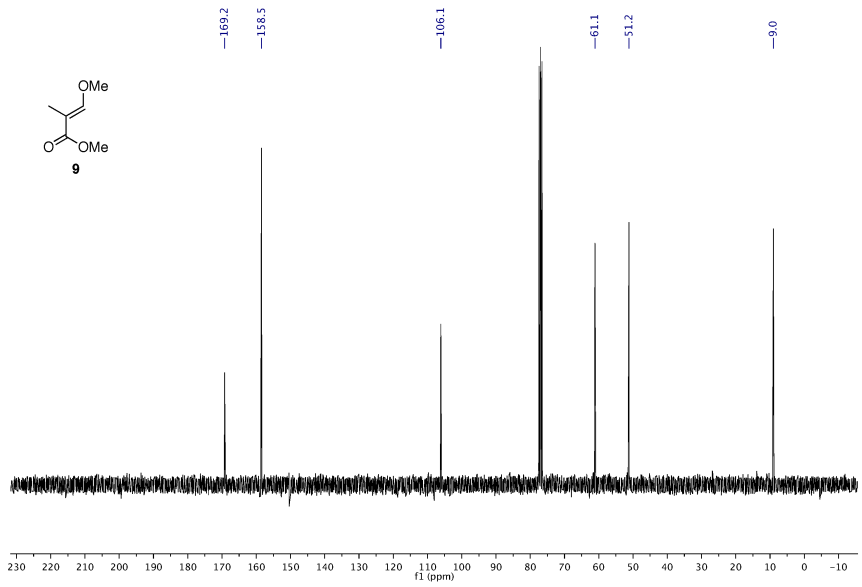
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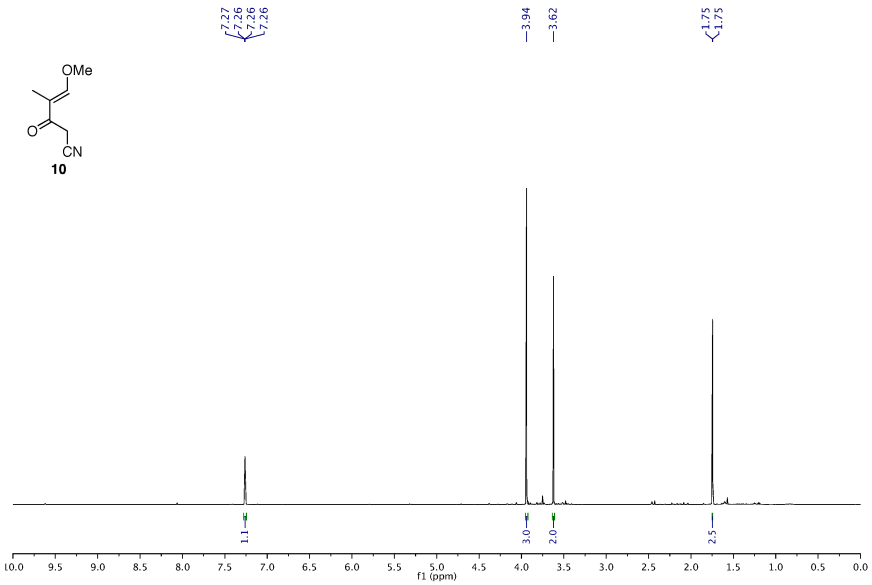
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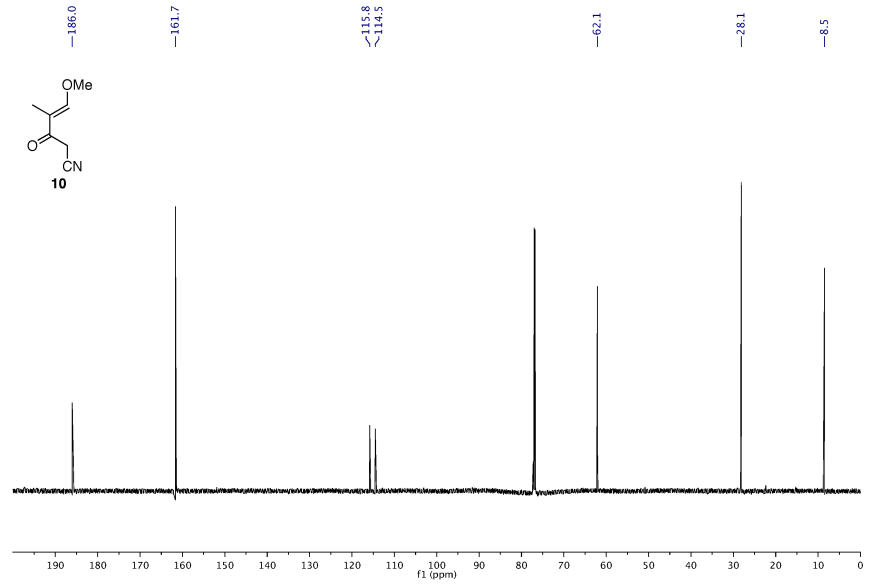
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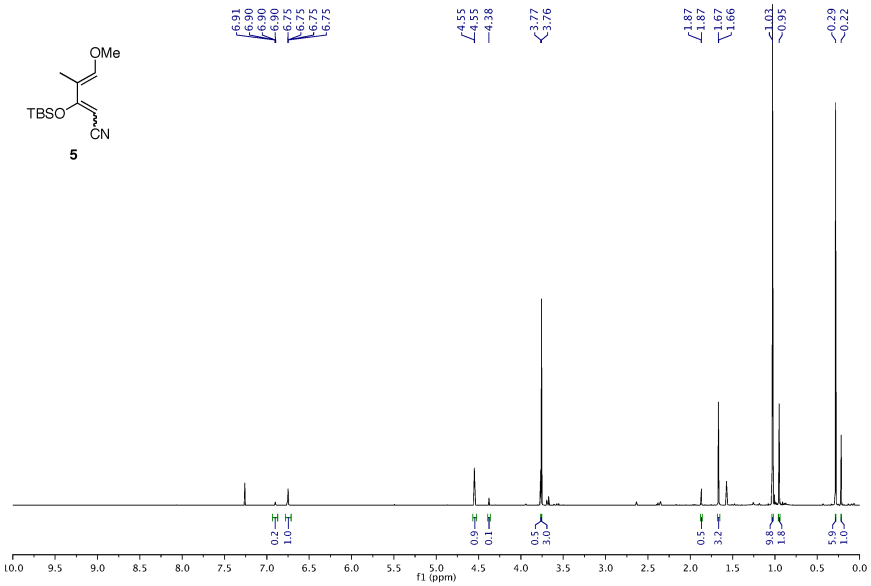
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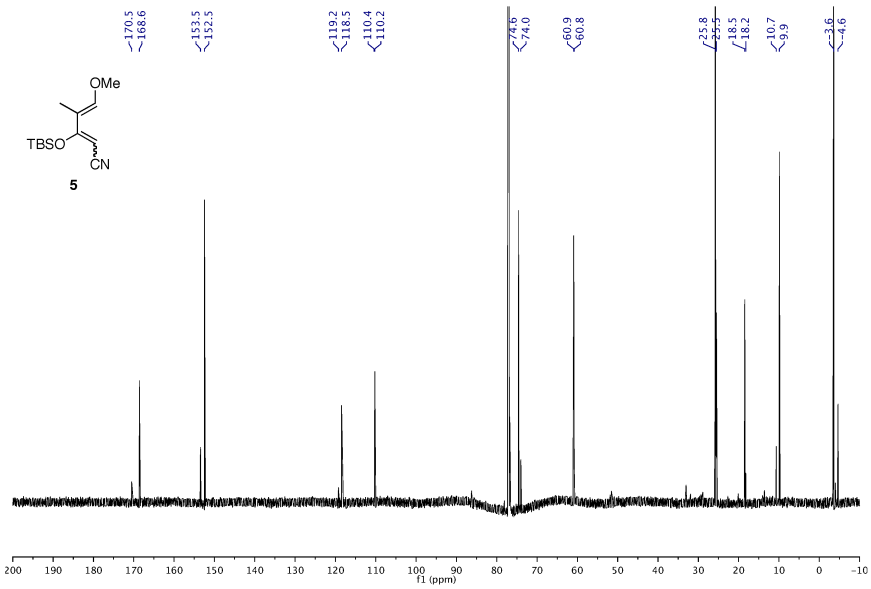
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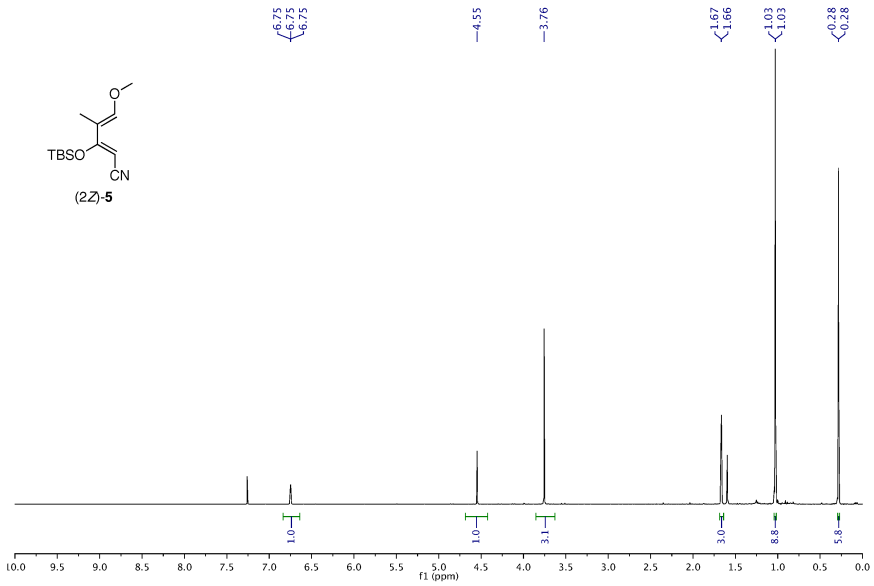
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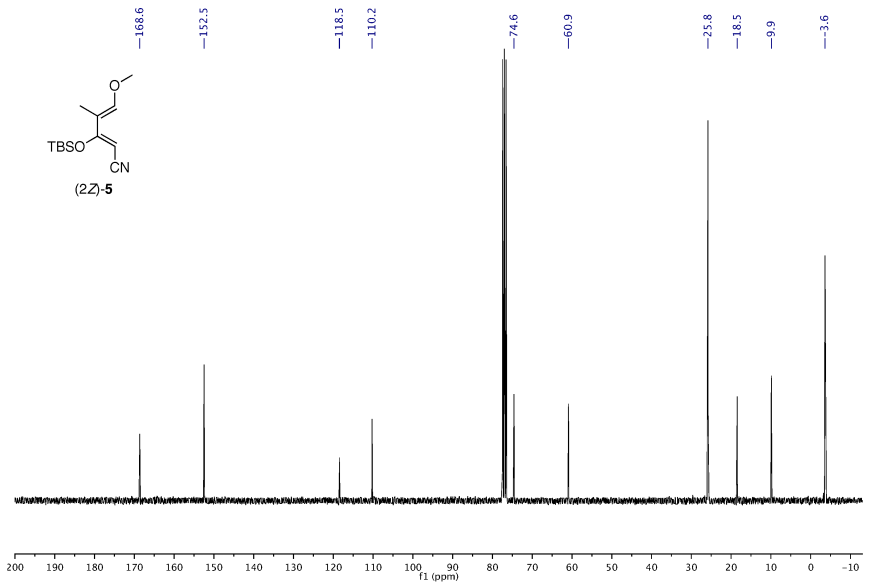
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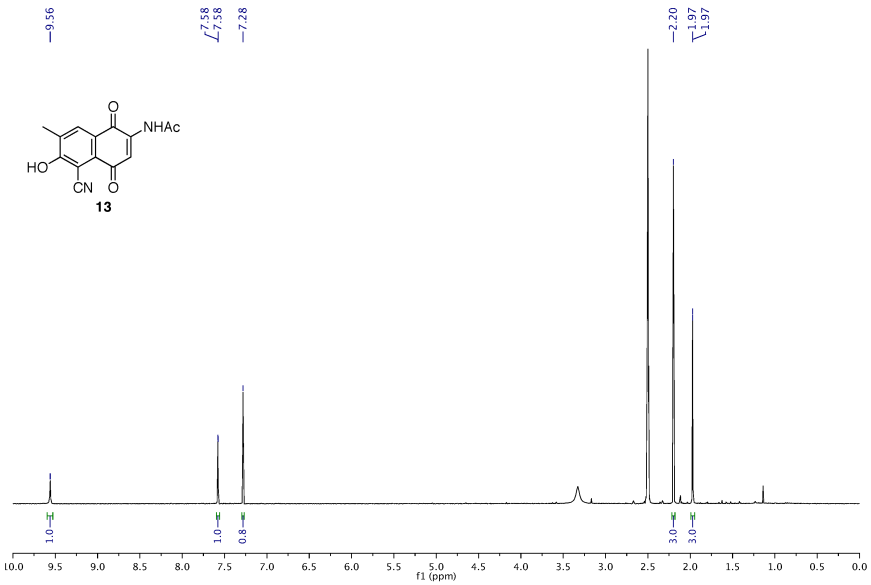
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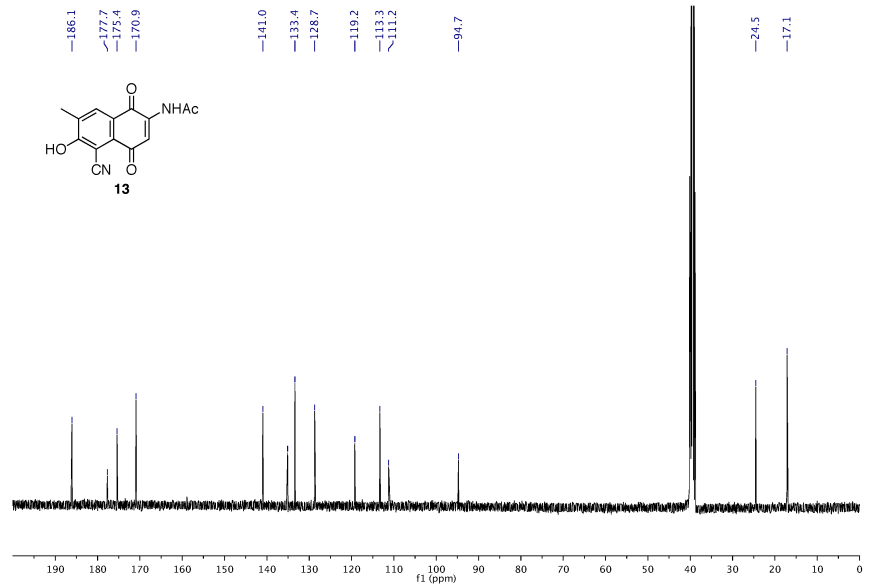
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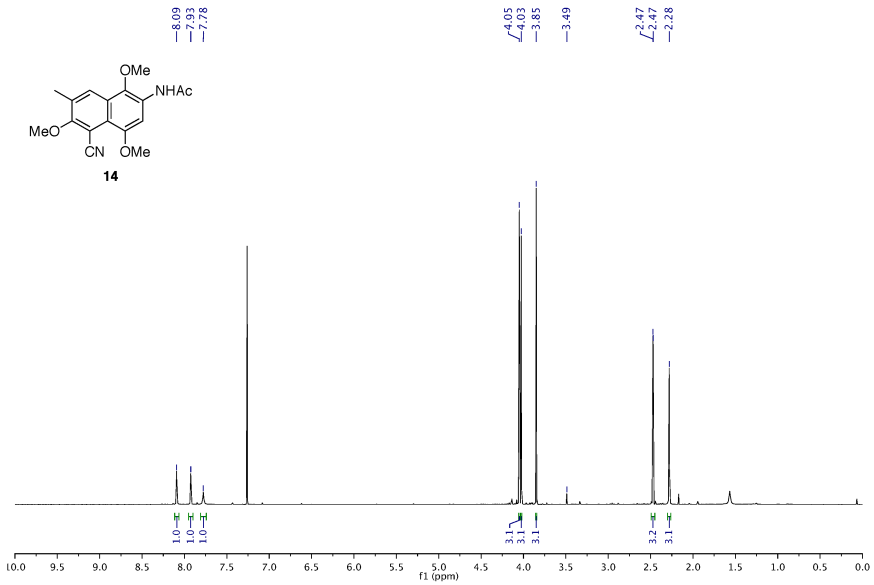
f1 (ppm)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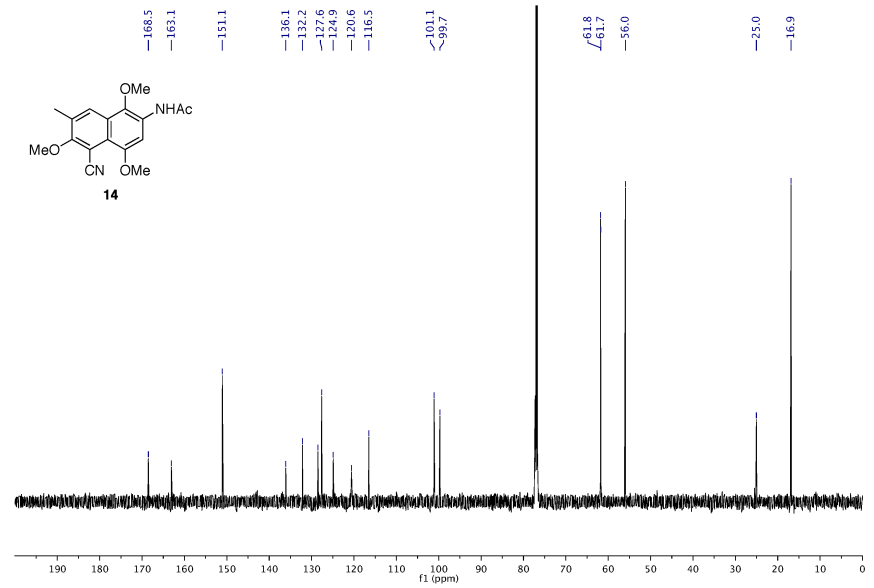
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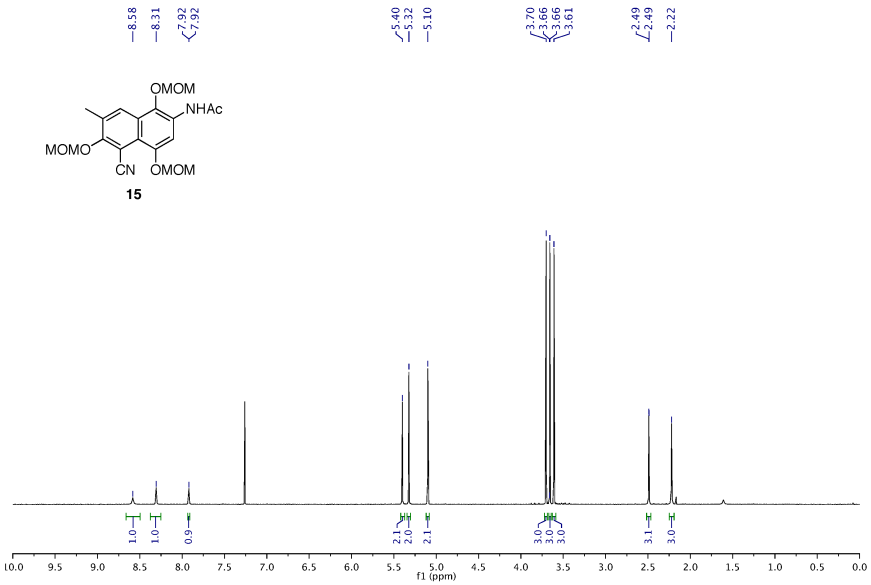
127.6
124.9
120.6
116.5
101.1
99.7
61.8
61.7
56.0
25.0
16.9
OMe
NHAc
MeO
CN
OMe
14
f1 (ppm)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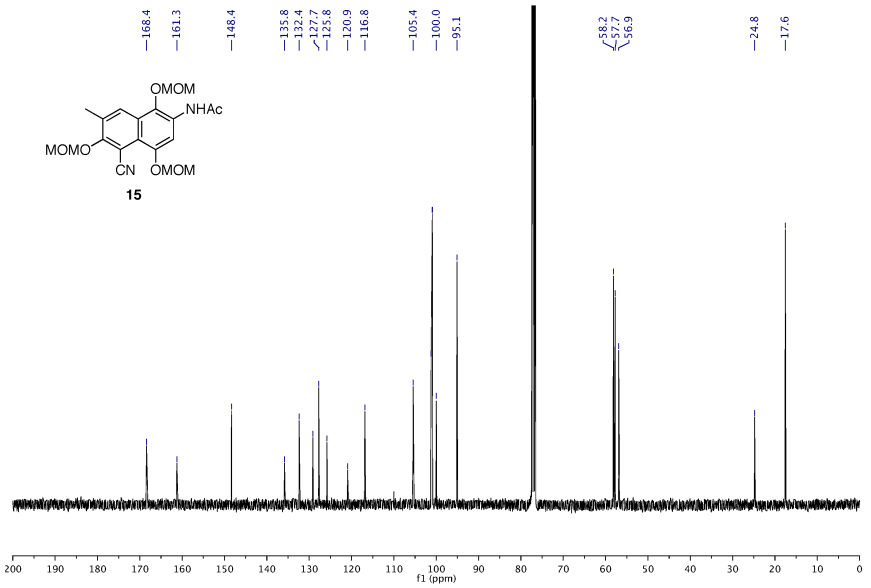
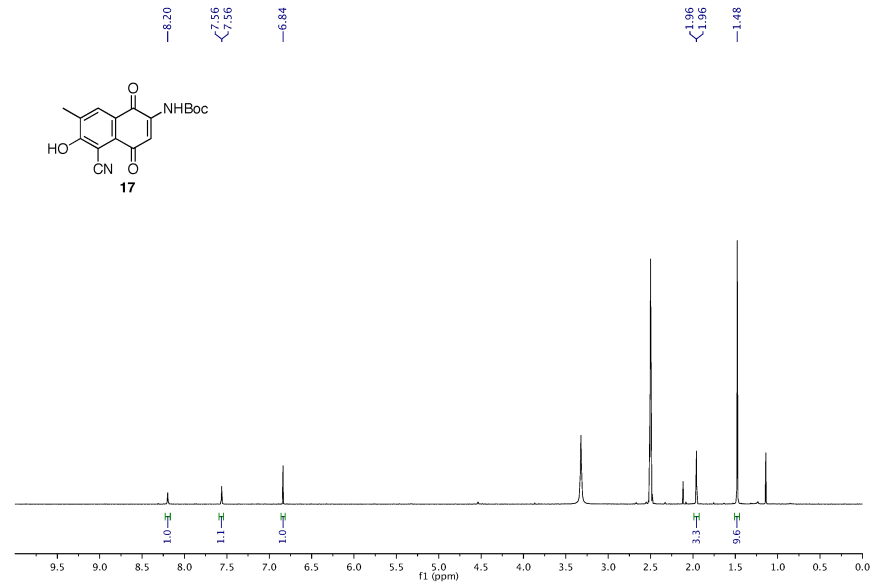
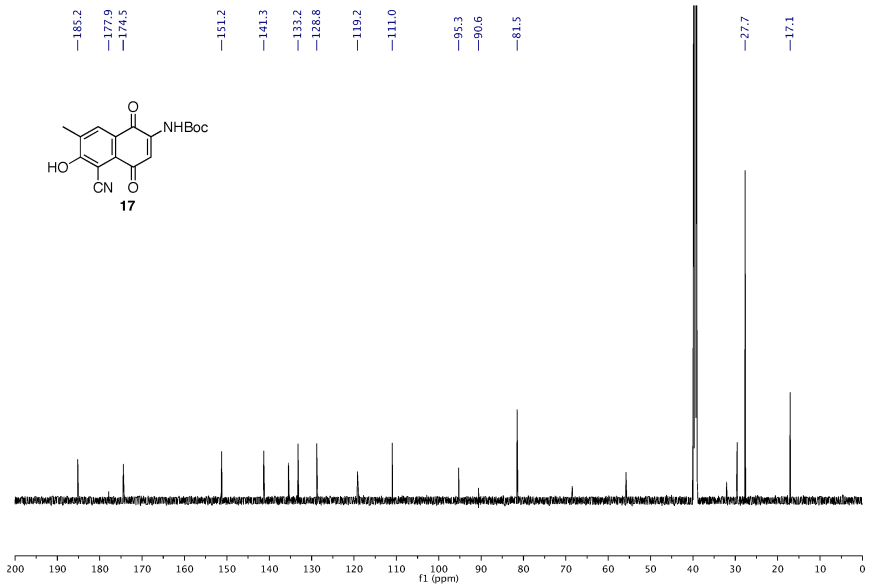
8.31
7.92
7.92
5.40
5.32
5.10
3.70
3.66
3.66
3.61
2.49
2.49
2.22
OMOM
NHAc
MOMO
CN
OMOM
15
1.0
1.0
0.9
2.1
2.0
2.1
3.0
3.0
3.0
3.1
3.0
f1 (ppm)


168.4
161.3
148.4
135.8
132.4
127.7
125.8
120.9
116.8
105.4
100.0
95.1
58.2
57.7
56.9
24.8
17.6
OMOM
NHAc
MOMO
CN
OMOM
15
f1 (ppm)


8.20
7.56
7.56
6.84
1.96
1.96
1.48
NHBoc
HO
CN
O
17
1.0
1.1
1.0
3.3
9.6
f1 (ppm)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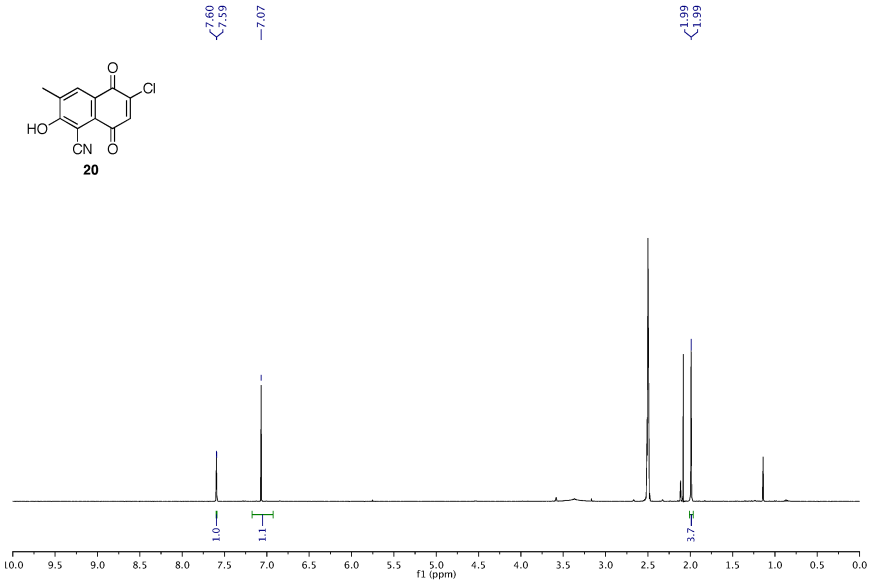
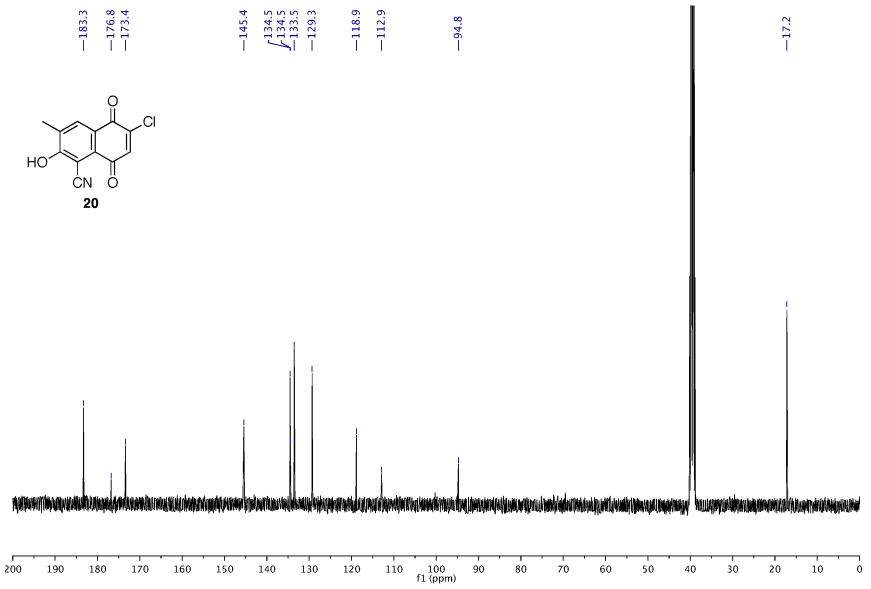
177.9
174.5
151.2
141.3
133.2
128.8
119.2
111.0
95.3
90.6
81.5
27.7
17.1
NHBoc
HO
CN
O
17
f1 (ppm)


7.60
7.59
7.07
1.99
1.99
O
Cl
HO
CN
O
20
1.0
1.1
3.7
f1 (ppm)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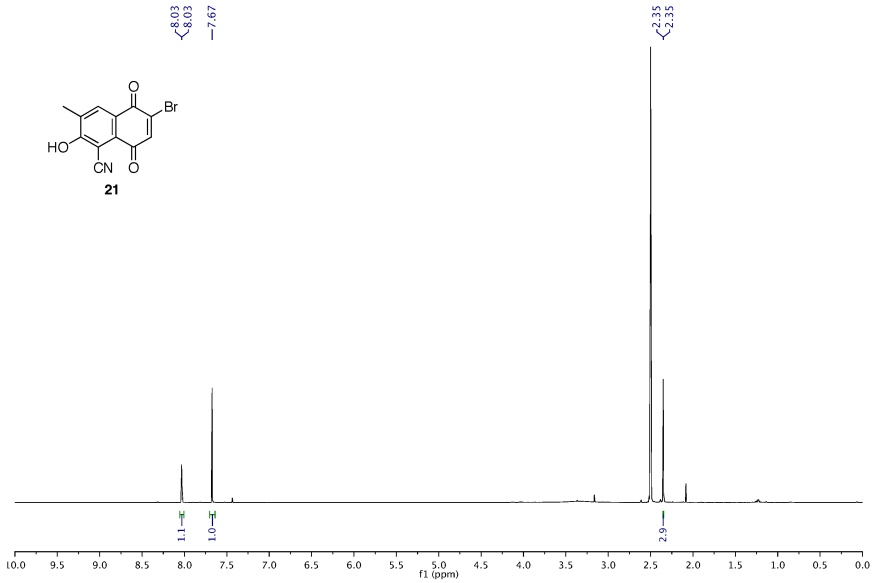
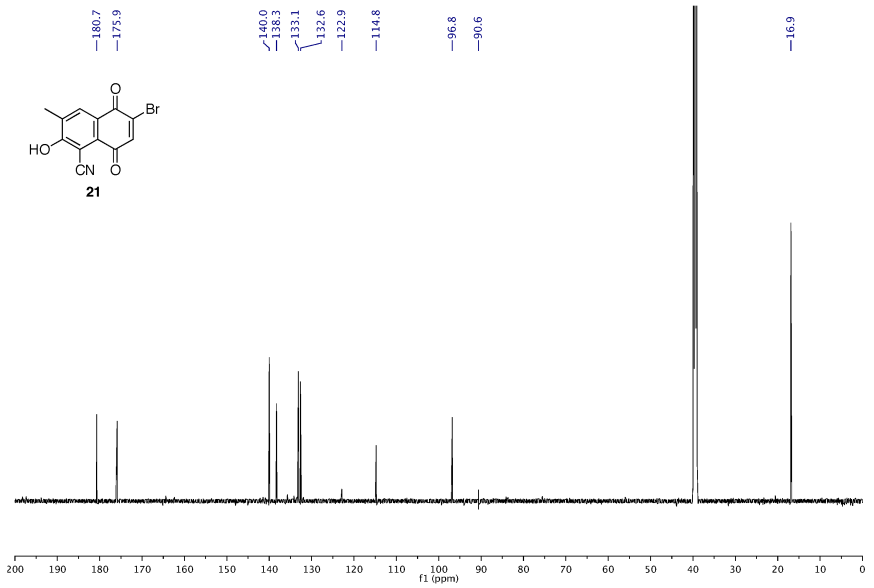
176.8
173.4
145.4
134.5
134.5
133.5
129.3
118.9
112.9
94.8
17.2
O
Cl
HO
CN
O
20
f1 (ppm)


8.03
8.03
7.67
2.35
2.35
Br
HO
CN
O
O
21
1.1
1.0
2.9
f1 (ppm)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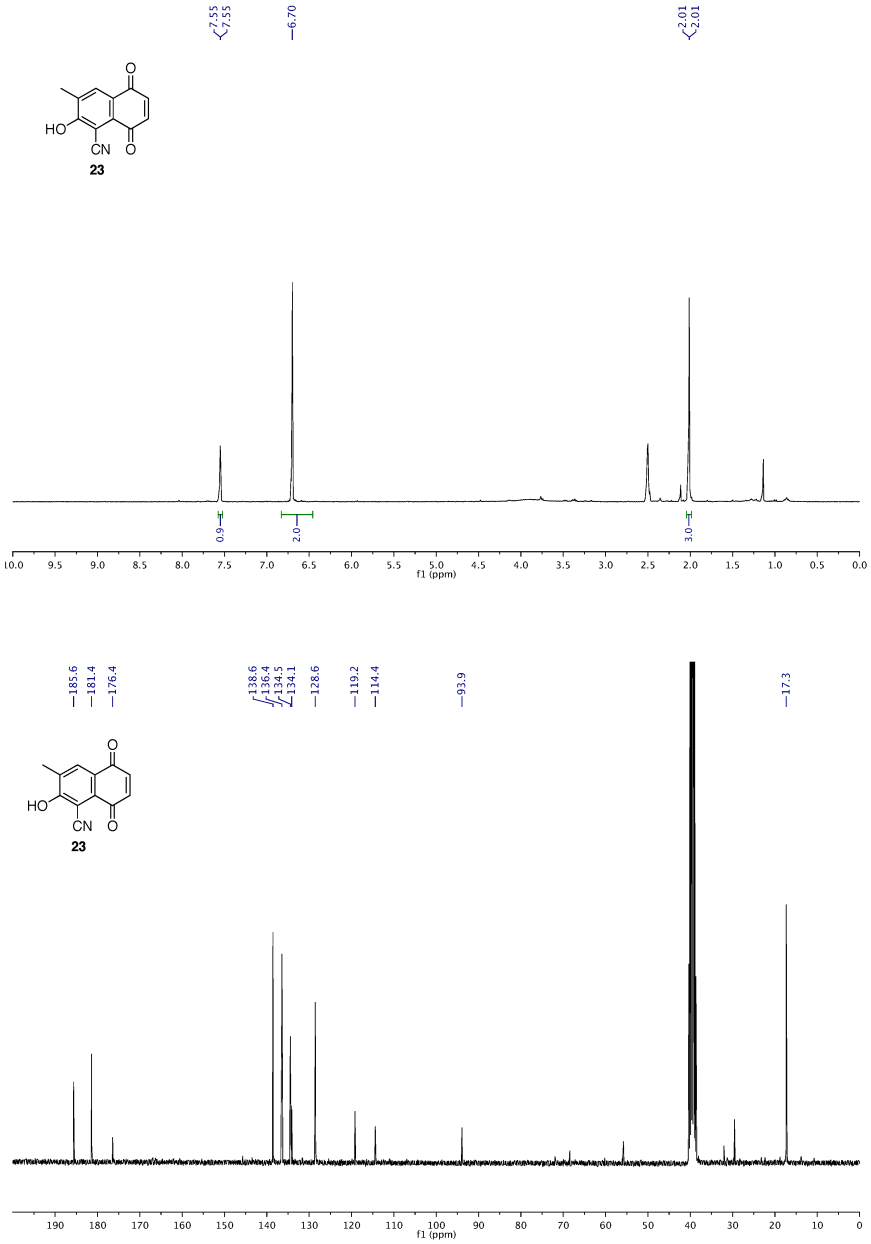
175.9
140.0
138.3
133.1
132.6
122.9
114.8
96.8
90.6
16.9
Br
HO
CN
O
O
21
f1 (ppm)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7.55
6.70
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2.01
HO
CN
O
O
23
0.9
2.0
3.0
f1 (ppm)
185.6
181.4
176.4
138.6
136.4
134.5
134.1
128.6
119.2
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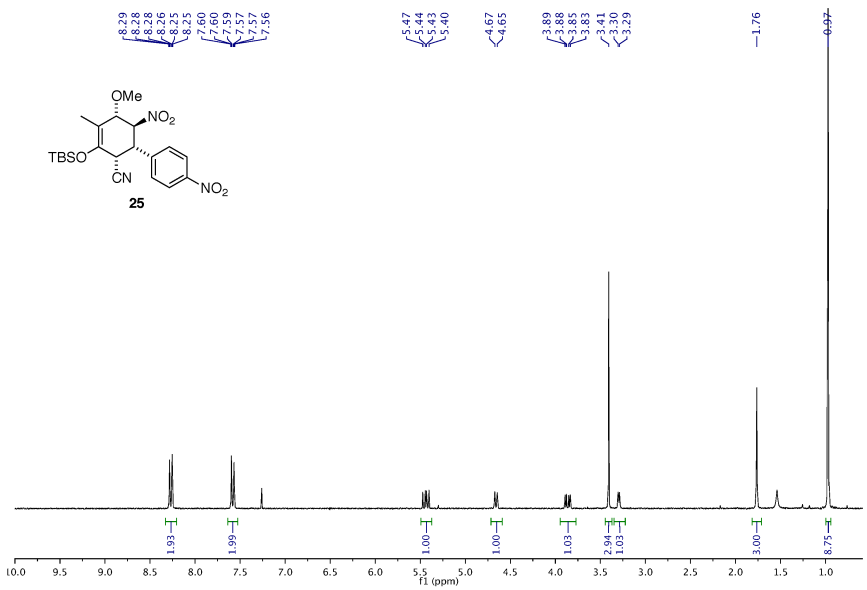
17.3
HO
CN
O
O
23
f1 (ppm)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8.28
8.28
8.26
8.25
8.25
7.60
7.60
7.59
7.57
7.57
7.56
5.47
5.44
5.43
5.40
4.67
4.65
3.89
3.88
3.85
3.83
3.41
3.30
3.29
1.76
0.97
OMe
NO2
TBSO
CN
NO2
25
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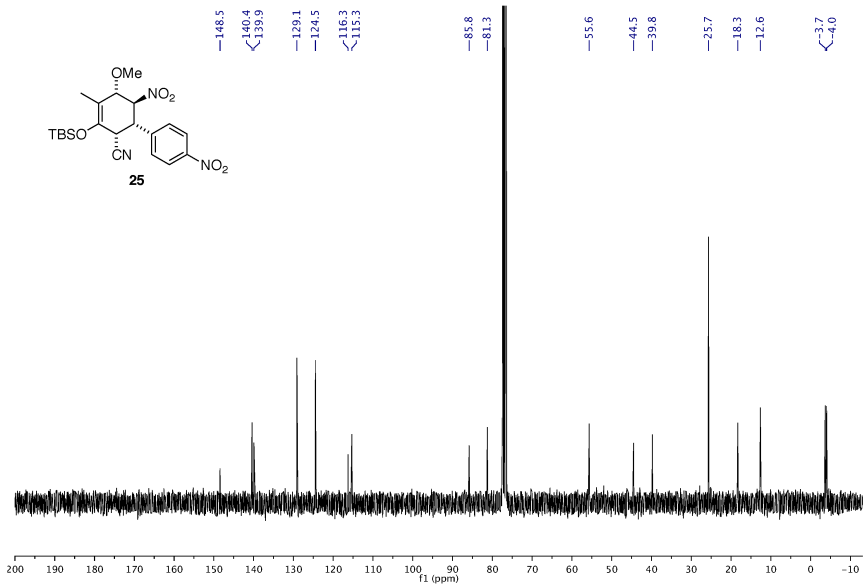
1.99
1.00
1.00
1.03
2.94
1.03
3.00
8.75
f1 (ppm)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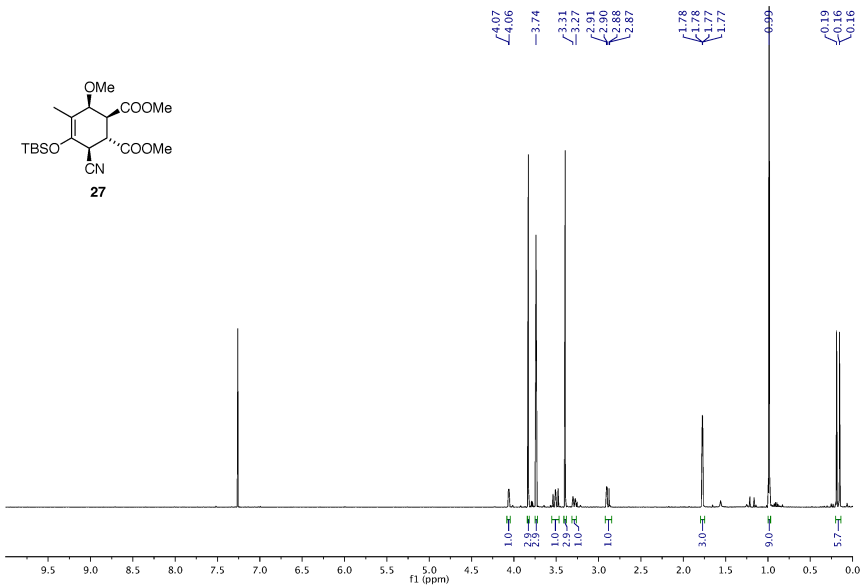
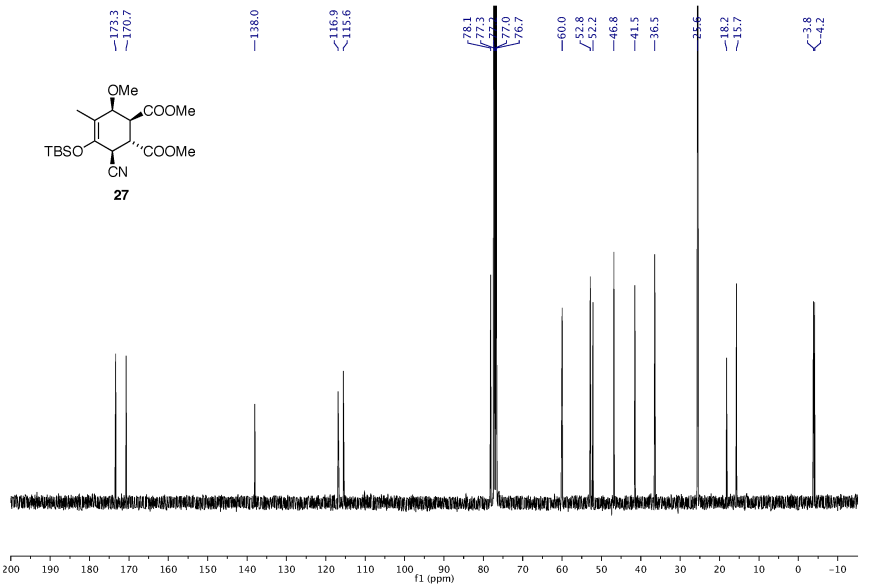
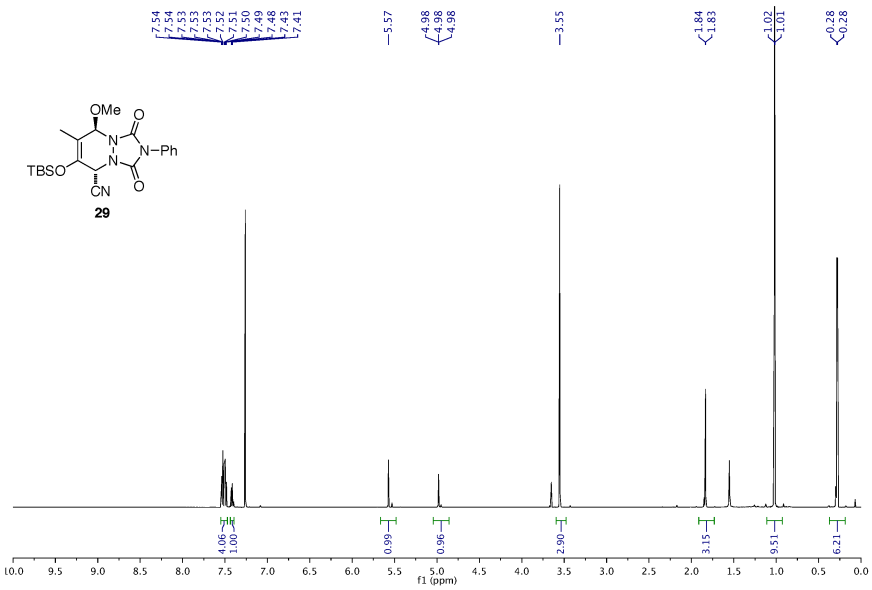
140.4
139.9
129.1
124.5
116.3
115.3
85.8
81.3
55.6
44.5
39.8
25.7
18.3
12.6
3.7
4.0
OMe
NO2
TBSO
CN
NO2
25
f1 (ppm)


OMe
COOMe
TBSO
COOMe
CN
27
4.07
4.06
3.74
3.31
3.27
2.91
2.90
2.88
2.87
1.78
1.78
1.77
1.77
0.99
0.19
0.16
0.16
f1 (ppm)


OMe
COOMe
TBSO
COOMe
CN
27
173.3
170.7
138.0
116.9
115.6
78.1
77.3
77.0
76.7
60.0
52.8
52.2
46.8
41.5
36.5
25.6
18.2
15.7
-3.8
-4.2
f1 (ppm)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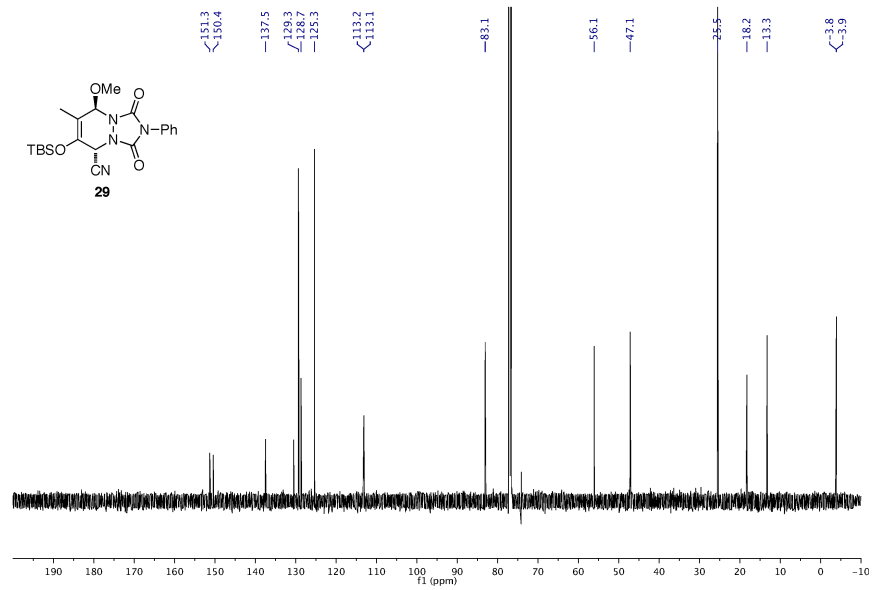
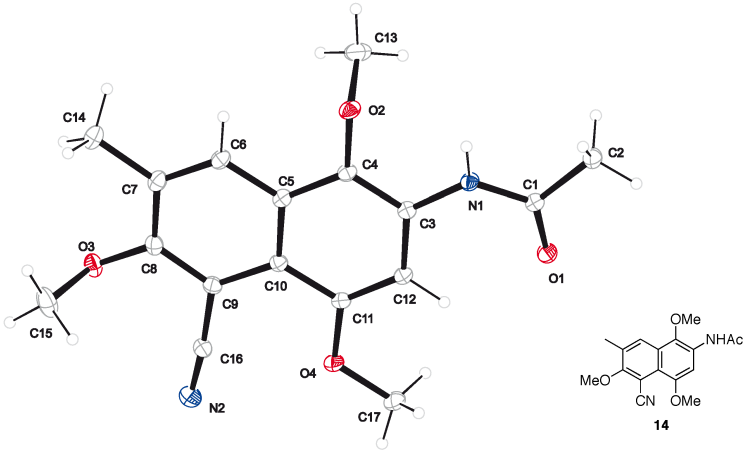
7.54
7.53
7.53
7.53
7.52
7.51
7.50
7.49
7.48
7.43
7.41
5.57
4.98
4.98
4.98
3.55
1.84
1.83
1.02
1.01
0.28
0.28
OMe
TBSO
CN
N–Ph
29
4.06
1.00
0.99
0.96
2.90
3.15
9.51
6.21
f1 (ppm)


151.3
150.4
137.5
129.3
128.7
125.3
113.2
113.1
83.1
56.1
47.1
25.5
18.2
13.3
-3.8
-3.9
OMe
TBSO
CN
N–Ph
29
f1 (ppm)

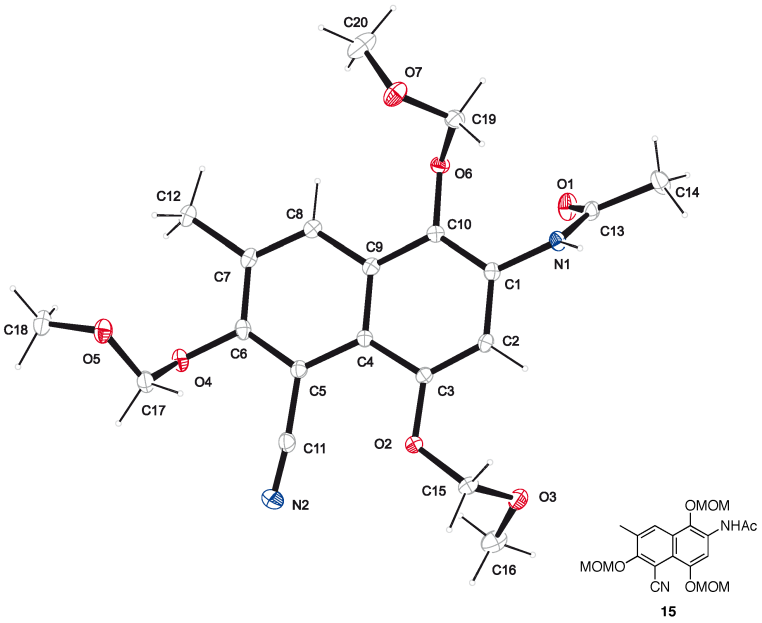
Crystal structures

Note: Crystallographic data for compounds **14**, **15**, **21**, **25**, **27** and **29** have been deposited at the Cambridge Crystallographic Data Centre (CCDC 859806, 859805, 859804, 864199, 859807, and 859808, respectively).

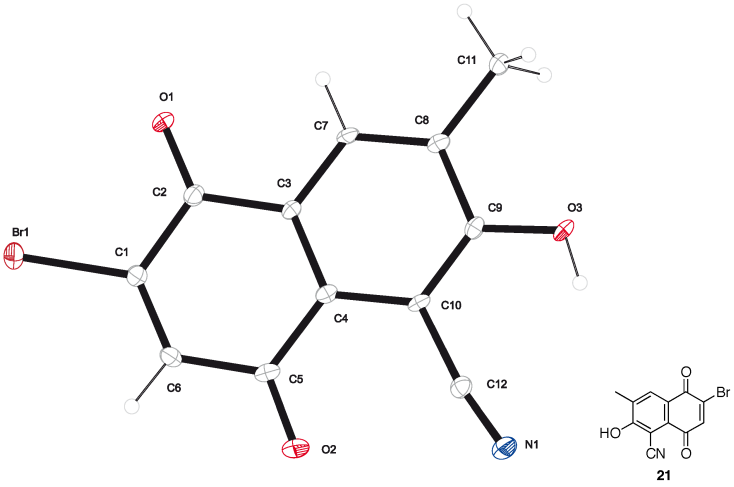
a) X-Ray structure of **14**:



b) X-Ray structure of **15**:

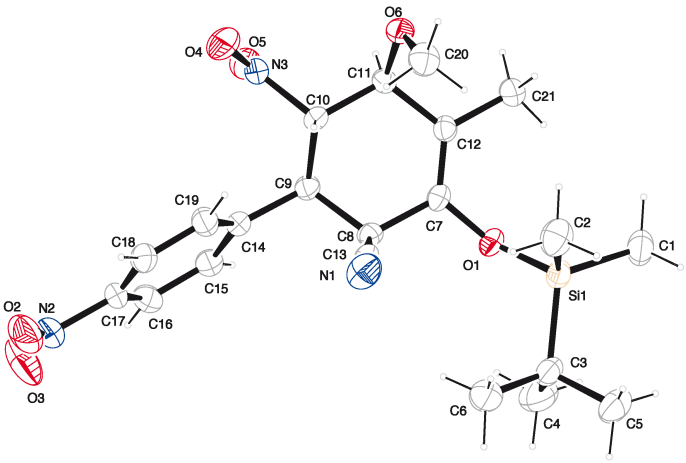


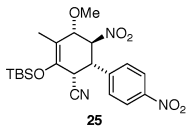
c) X-Ray structure of **21**:



21

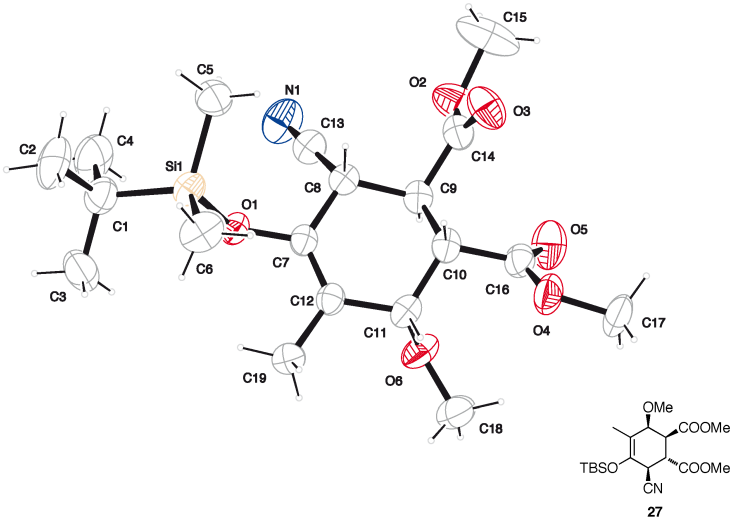
d) X-Ray structure of **25**:



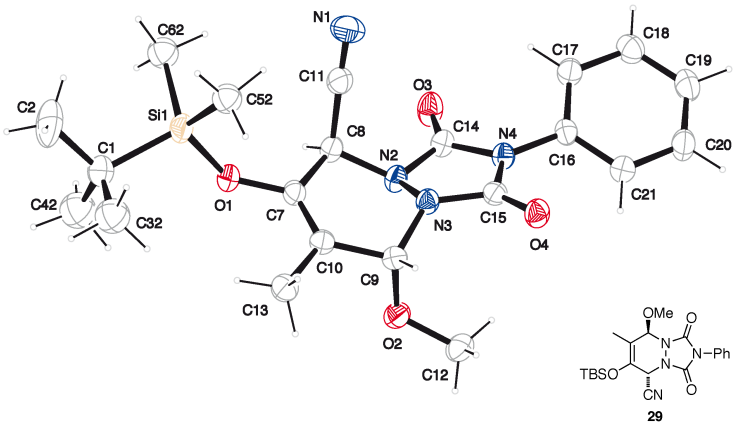


25

e) X-Ray structure of **27**:



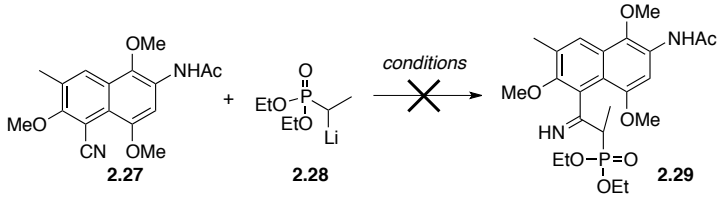
f) X-Ray structure of **29**:



1.3 Unpublished Results

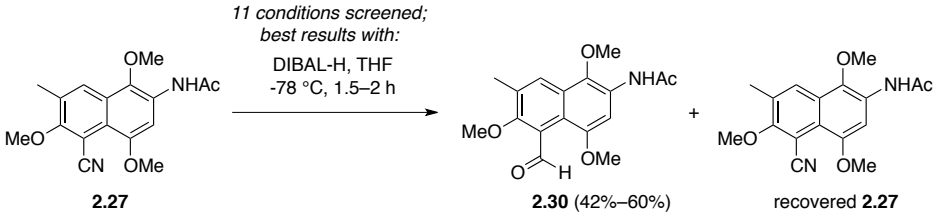
1.3.1 Attempts to the Synthesis of **2.17**

With the methyl protected hydroquinone **2.27** in hand, the attachment of phosphonate **2.23** to the cyano group was attempted (Scheme 5). To this end, diethyl ethylphosphonate (**2.23**) was deprotonated using *n*-BuLi in THF at -78 °C and the resulting lithiated species **2.28** was reacted with cyano-naphthalene **2.27**. However, only starting material was recovered despite the large excess of **2.28** that was used. We reasoned that the cyano group was not electrophilic enough for an attack to occur and hence tried to increase its reactivity by the addition of Lewis-acids.[31] Unfortunately, no product formation was observed in the presence of $AlCl_3$ or $BF_3{\cdot}OEt_2$, even when the reaction mixture was refluxed for extended reaction times.



Scheme 5. Attempted addition of a lithiated phosphonate **2.28** into cyanonaphthalene **2.27**.

We next tried to reduce the cyano group to a formyl group in order to increase its electrophilicity (Scheme 6). To this end, several conditions were screened, albeit with little success. In most cases, either decomposition was observed or a mixture of starting material **2.27** and deacetylated starting material was isolated. Reduction was only achieved when **2.27** was reacted with 5.5–6 equivalents of DIBAL-H at -78 °C for 1.5–2 h. However the yields were only moderate (42%–60%; determined by NMR) and the reaction was not reproducable. Furthermore, the product could not be separated from the remaining starting material.

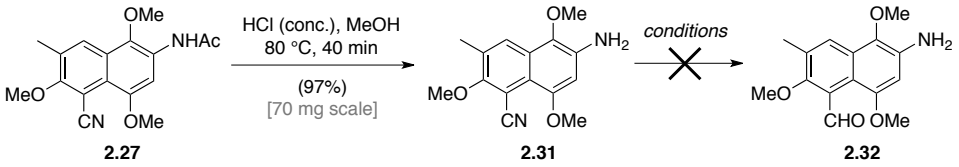


Scheme 6. Attempted reduction of the cyano group present in **2.27**.

To exclude the possibility that the acetyl group was responsible for the difficulties with the reduction, it was cleaved by treatment with conc. HCl in MeOH and the resulting free amine

[31] McKenna, M. T.; Proctor, G. R.; Young, L. C.; Harvey, A. L. *J. Med. Chem.* **1997**, *40*, 3516–3523.

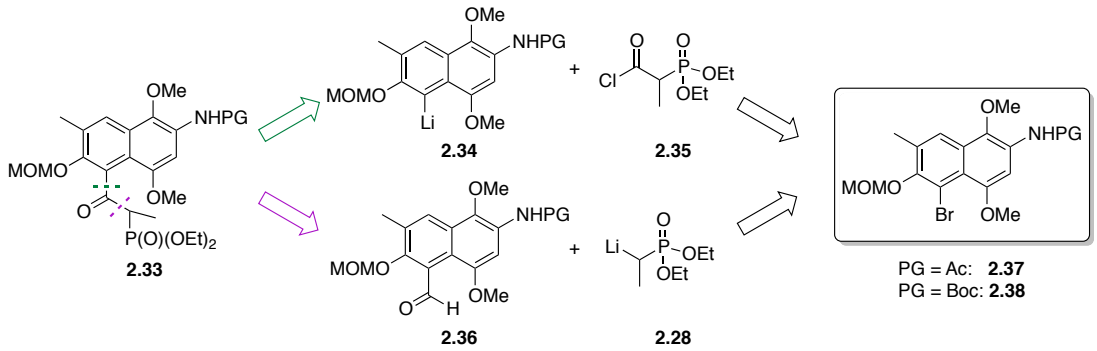
2.31 was exposed to the reduction conditions (Scheme 7). Unfortunately, none of the attempted reductions gave any product.



Scheme 7. Cleavage of the acetate in **2.27** and subsequent attempts to reduce the cyano group.

1.3.2 2nd Approach to the Naphthalene Core

Because **2.27** could not be further elaborated into the desired phosphonate **2.17**, we devised a new strategy for the synthesis of **2.33**, shown in Scheme 8. Inspired by Roush´s synthesis of (+)-damavaricin D,[32] we reasoned that the challenging introduction of the phosphonate on the naphthalene should be feasible by either acylation of lithiated intermediate **2.34** using acyl-phosphonate **2.35** or nucleophilic attack of lithiated phosphonate **2.28** on formyl naphthalene **2.36** and subsequent oxidation. Both, **2.34** and **2.36** could in turn be traced back to amino-protected bromonaphthalenes **2.37** and **2.38**, both of which are key intermediates in our revised strategy (Scheme 8).



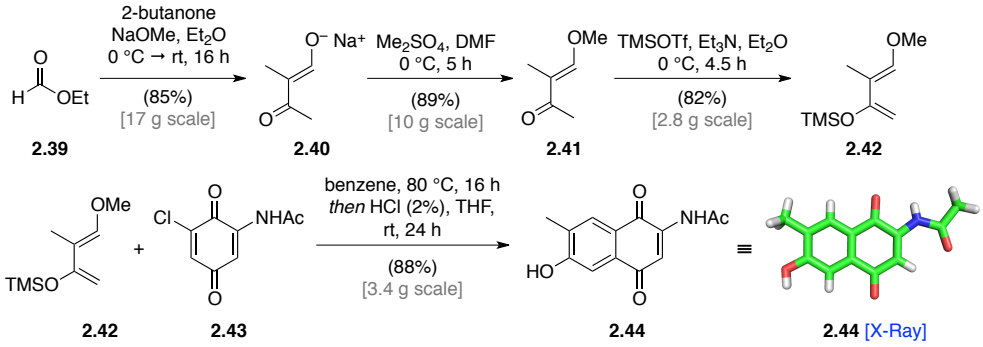
Scheme 8. Revised strategy to **2.33** starting from a common building block. The two different retrosynthetic cuts and pathways are highlighted in colour.

In the following two sections, the syntheses of the two different protected bromonaphthalenes **2.37** and **2.38** are described.

[32] Roush, W. R.; Coffey, D. S.; Madar, D. J. *J. Am. Chem. Soc.* **1997**, *119*, 11331–11332.

1.3.2.1 Synthesis of Acetyl-protected Bromonaphthalene 2.37

The synthesis of acetyl bromonaphthalene **2.37** commenced with the preparation of the known methyl-Danishefsky diene **2.42** (Scheme 9).[33] Thus, 2-butanone was deprotonated using sodium methoxide and reacted with ethyl formate (**2.39**) to give sodium enolate **2.40**,[34] which was methylated to afford enol ether **2.41** in very good yield over 2 steps. Compound **2.41** was then transformed into the corresponding diene **2.42** by treatment with triethyl amine and TMSOTf in Et_2O in 82% yield.[35] After purification of the crude diene by distillation, it was ready to be used in the planned Diels-Alder reaction. The required dienophile, 6-chloro-2-acetamidobenzoquinone (**2.43**), was synthesized in three steps from commercially available 2,4-dichloro-6-nitrophenol following a procedure by Kelly and coworkers.[36] With multigram quantities of the diene **2.42** and the dienophile **2.43** in hand, the stage was set for the crucial Diels-Alder reaction. Thus, **2.42** and **2.43** were combined in a pressure tube and heated at 80 °C for 16 h. Subsequent aromatization was achieved by treatment of the crude with HCl (2%) in THF to give aminonaphthoquinone **2.44** in 88% yield as a single regioisomer, the structure of which was confirmed by X-ray crystallographic analysis (Scheme 9).

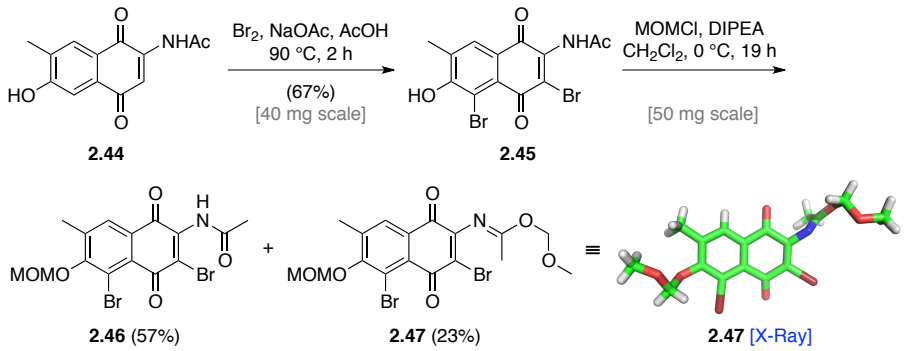


Scheme 9. Synthesis and X-ray structure of aminonaphthoquinone **2.44**.

For the next step, a method for the regioselective monobromination of **2.44** was sought. Since there was no precedence for the targeted transformation, suitable conditions had to be found. Reaction of **2.44** with bromine in the presence of AcOH/NaOAc afforded the dibrominated compound **2.45** depicted in Scheme 10. Although this reaction did not give the desired product, the result was quite interesting and provided an important clue. During the reaction,

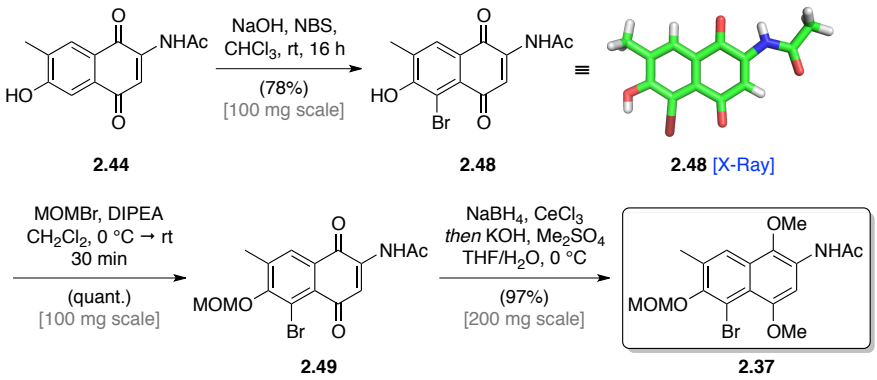
[33] (a) Barrett, A. G. M.; Carr, R. A. E.; Attwood, S. V.; Richardson, G.; Walshe, N. D. A. *J. Org. Chem.* **1986**, *51*, 4840–4856; (b) Clive, D.; Bergstra, R. J. *J. Org. Chem.* **1991**, *56*, 4976–4977; (c) Miyashita, M.; Yamasaki, T.; Shiratani, T.; Hatakeyama, S.; Miyazawa, M.; Irie, H. *Chem. Comm.* **1997**, *18*, 1787–1788.
[34] Gupta, R. C.; Larsen, D. S.; Stoodley, R. J.; Slawin, A. M. Z.; Williams, D. J. *J. Chem. Soc. Perkin Trans. 1* **1989**, 739–749.
[35] Yamashita, Y.; Saito, S.; Ishitani, H.; Kobayashi, S. *J. Am. Chem. Soc.* **2003**, *125*, 3793–3798.
[36] Kelly, T. R.; Echavarren, A.; Behforouz, M. *J. Org. Chem.* **1983**, *48*, 3849–3851.

the bromide anion, which is formed after initial reaction of the arene with Br_2, proved sufficiently nucleophilic to undergo a Michael-addition to the α,β-unsaturated system of the quinone. As such, we believe that it is feasible to install the required C30-substituents of the different naphthomycines by simply reacting naphthomycin E (**2.8**) with the corresponding nucleophile. Although compound **2.45** was not further carried on through the synthesis, it was used to test the subsequent MOM protection. Interestingly, when **2.45** was reacted with MOMCl and DIPEA overnight, in addition to the expected MOM-ether **2.46**, compound **2.47** was identified and its structure was verified through X-ray crystallography.



Scheme 10. Interesting results from initial bromination and MOM protection attempts.

In order to achieve the key monobromination *ortho* to the hydroxy group present in **2.44**, several bromination reagents, solvents and bases were screened. This resulted in the finding that reaction of **2.44** with *N*-bromosuccinimide and NaOH in $CHCl_3$ cleanly yielded the target compound **2.48** in 78% yield. The structure of this monobrominated aminonaphthoquinone was subsequently proven by single X-ray crystallographic analysis (Scheme 11). Protection of phenol **2.48** as a MOM ether was accomplished quantitatively by treatment with bromomethyl methyl ether and Hünig's base. The resulting naphthoquinone **2.49** was reduced using sodium borohydride and $CeCl_3$ in a THF/water mixture and the resulting air-sensitive 1,4-dihydroquinone was methylated *in situ* by treatment with dimethyl sulfate and KOH as a base under exclusion of oxygen. This one-pot reduction-methylation procedure afforded the desired 1,4-di-*O*-methyldihydroquinone **2.37** in excellent yield.

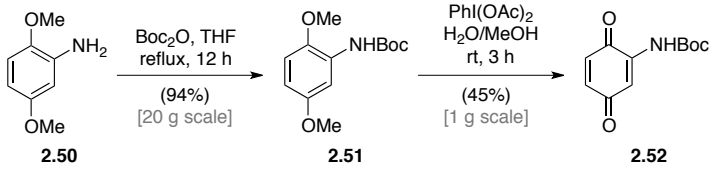


Scheme 11. Synthesis of the key acetyl protected bromonaphthalene **2.37**.

As such, an efficient route for the key intermediate **2.37** was developed which allowed for its synthesis in only seven steps (longest linear sequence) from commercially available starting materials with an overall yield of 47%.

1.3.2.2 Synthesis of Boc-protected Bromonaphthalene 2.38

Having worked out a reliable and scalable route to **2.37**, the Boc protected analogue **2.38** was next targeted. To this end, 2-*tert*-butoxycarbonylamino-1,4-benzoquinone (**2.52**) was first prepared according to a recently published procedure (Scheme 12).[37] This included Boc-protection of 2,5-dimethoxyaniline (**2.50**) to give **2.51** followed by oxidation using $PhI(OAc)_2$ to afford **2.52**. Whilst the first step of this protocol could easily be scaled up to decagram quantities, it should be noted that the yield of the oxidation step rapidly decreased when the reaction was scaled up.

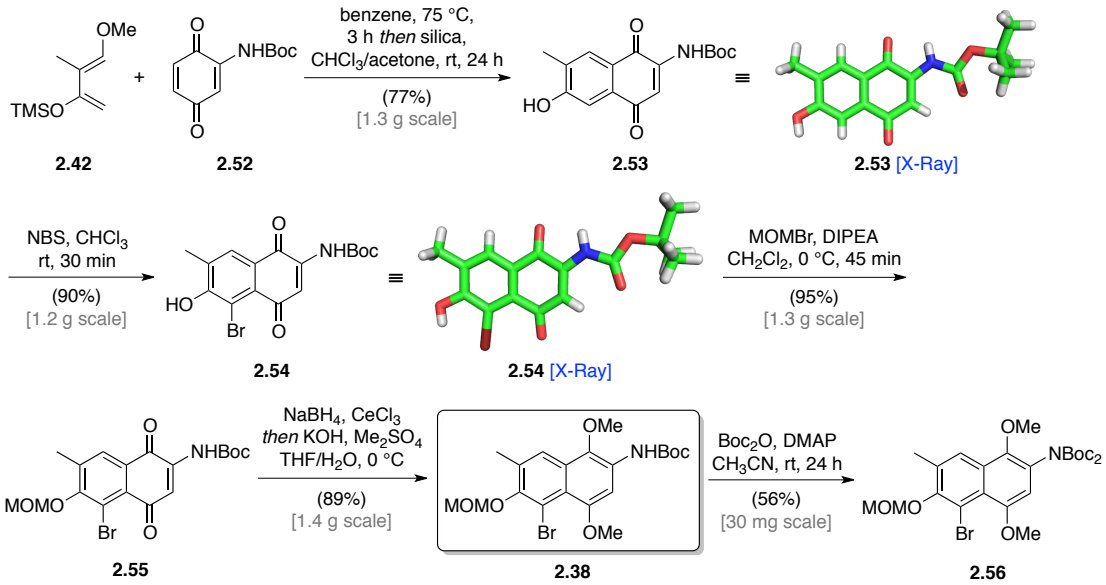


Scheme 12. Synthesis of Boc-protected aminoquinone **2.52.**

With sufficient material in hand, the synthesis of **2.56** was carried out as shown in Scheme 13. It commenced with the Diels-Alder reaction between diene **2.42** and quinone **2.52**, which was performed in a pressure tube at 75 °C. In this case however, a further oxidation step was necessary to aromatize the Diels-Alder adduct. This could be effected when a suspension of the crude product mixture and silica gel was stirred in $CHCl_3$/acetone and subsequently pushed through a silica gel column. As a result, aminonaphthoquinone **2.53** was obtained in

[37] Nawrat, C. C.; Lewis, W.; Moody, C. J. *J. Org. Chem.* **2011**, *76*, 7872–7881.

77% as a single isomer, whose structure was unambiguously confirmed through X-ray crystallographic analysis. Selective monobromination of Boc-protected aminonaphthoquinone, applying optimized conditions, gave compound **2.54** in very good yield. Again, the structure of the product was verified by X-ray crystallographic analysis. The next 2 steps were analogous to the route shown in Scheme 11. Conversion of bromophenol **2.54** into the corresponding MOM-ether **2.55** proceeded in almost quantitative yield. Subsequent reduction of **2.55** using sodium borohydride and *in situ* protection of the formed hydroquinone as the dimethyl ether using dimethyl sulfate (see above) provided key Boc-protected bromonaphthalene **2.38** in 89% yield. Since at this point, it was not clear if the NH-proton would be disadvantageous for the planned lithiation of **2.38**, it was shown that **2.38** could further be protected as the Di-Boc-bromonaphthalene **2.56** using Boc_2O and DMAP (56% unoptimized yield).



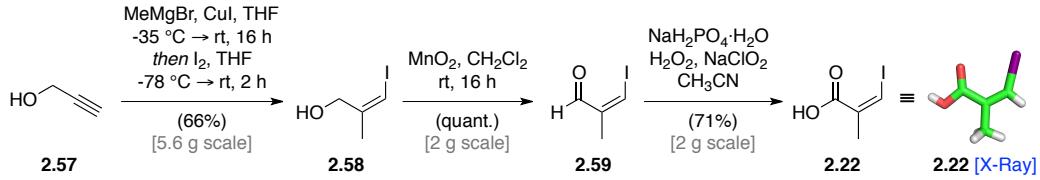
Scheme 13. Efficient synthesis of Boc-protected bromonaphthalene **2.38** starting from the known building blocks **2.42** and **2.52**.

In summary, Boc-protected bromonaphthalene could be synthesized on a gram scale starting from the known building blocks **2.42** and **2.52** in four steps with an overall yield of 59%.

1.3.2.3 Synthesis of (*Z*)-vinyl iodide 2.22

(*Z*)-3-iodo-2-methylpropenoic acid **2.22**, which was later intended to be coupled to the amino group of the naphthalene building block (see section 1.1.6), was synthesized in three steps as shown in Scheme 14. Cu-catalyzed methylmagnesation of propargyl alcohol (**2.57**) and trapping of the reactive intermediate with iodine, using a modified version of a reported

protocol,[38] yielded vinyl alcohol **2.58** in 66% yield. Allylic oxidation using manganese dioxide furnished sensitive aldehyde **2.59**, which was directly used in the next step without purification. Finally, Pinnick oxidation of **2.59** was carried out following a procedure by Liu and Negishi[39] and afforded the desired (*Z*)-vinyl iodide **2.22**, the structure of which was unambiguously confirmed by X-ray crystallographic analysis.



Scheme 14. Synthesis and X-ray structure of (*Z*)-vinyl iodide **2.22**.

[38] Hiroya, K.; Takuma, K.; Inamoto, K.; Sakamoto, T. *Heterocycles* **2009**, *77*, 493–505.
[39] Liu, F.; Negishi, E. *J. Org. Chem.* **1997**, *62*, 8591–8594.

2 Towards the Total Synthesis of Divergolides C and D

2.1 Isolation, Structure and Biological Activities

In 2011, Hertweck *et al.* reported the isolation, structural elucidation and biological activities of four novel ansamycins, which were named divergolides A–D (**2.60**–**2.61** and **2.3**–**2.4**).[40] They were isolated from an endophyte of the mangrove tree *Bruguiera gymnorrhiza*. This small tree is widely distributed in the tropics and grows on the seaward side of estuaries in mud. The bark and the root of *B. gymnorrhiza* is used to treat hemostasia, throat inflammation and diarrhea in traditional Chinese medicine.[41]

Hertweck´s group was able to elucidate the structures of the divergolides A–D based on careful 2D-NMR analysis and high resolution mass spectrometry. In addition, the absolute configuration of **2.60** was confirmed by X-ray crystallography and CD spectroscopy.

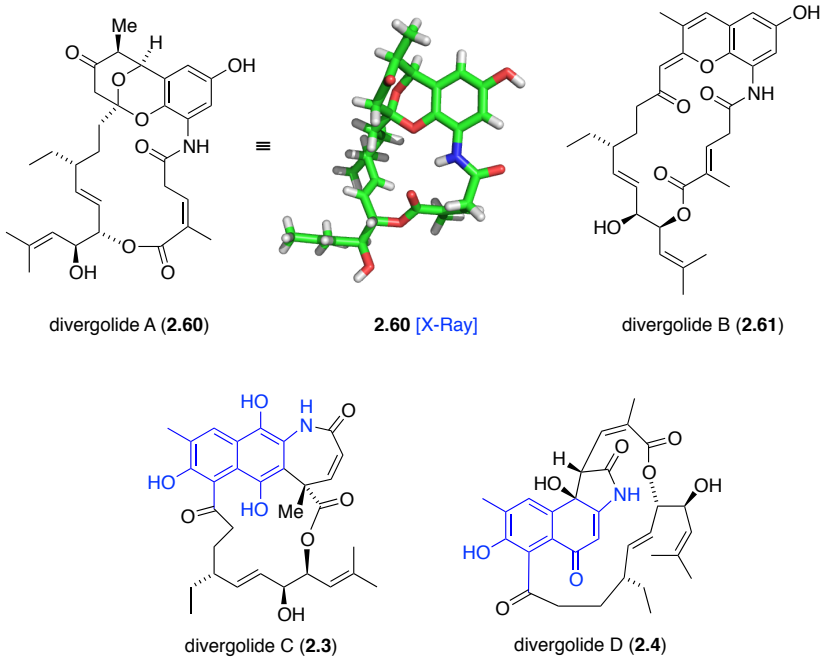


Figure 9. Structures of the recently isolated novel ansamycins divergolides A–D (**2.60**–**2.61** and **2.3**–**2.4**). Divergolides C (**2.3**) and D (**2.4**) share a common naphthoquinone core (highlighted in blue), which is also present in the naphthomycins (see section 1.1.2).

In order to evaluate the biological activities of the divergolides, they were tested in a number of antimicrobial, antiproliferative and cytotoxic assays. This screening revealed that

[40] Ding, L.; Maier, A.; Fiebig, H.-B.; Görls, H.; Lin, W.-H.; Peschel, G.; Hertweck, C. *Angew. Chem. Int. Ed.* **2011**, *50*, 1630–1634.
[41] Han, L.; Huang, X. S.; Sattler, I.; Dahse, H. M.; Fu, H. Z.; Lin, W. H.; Grabley, S. *J. Nat. Prod.* **2004**, *67*, 1620–1623.

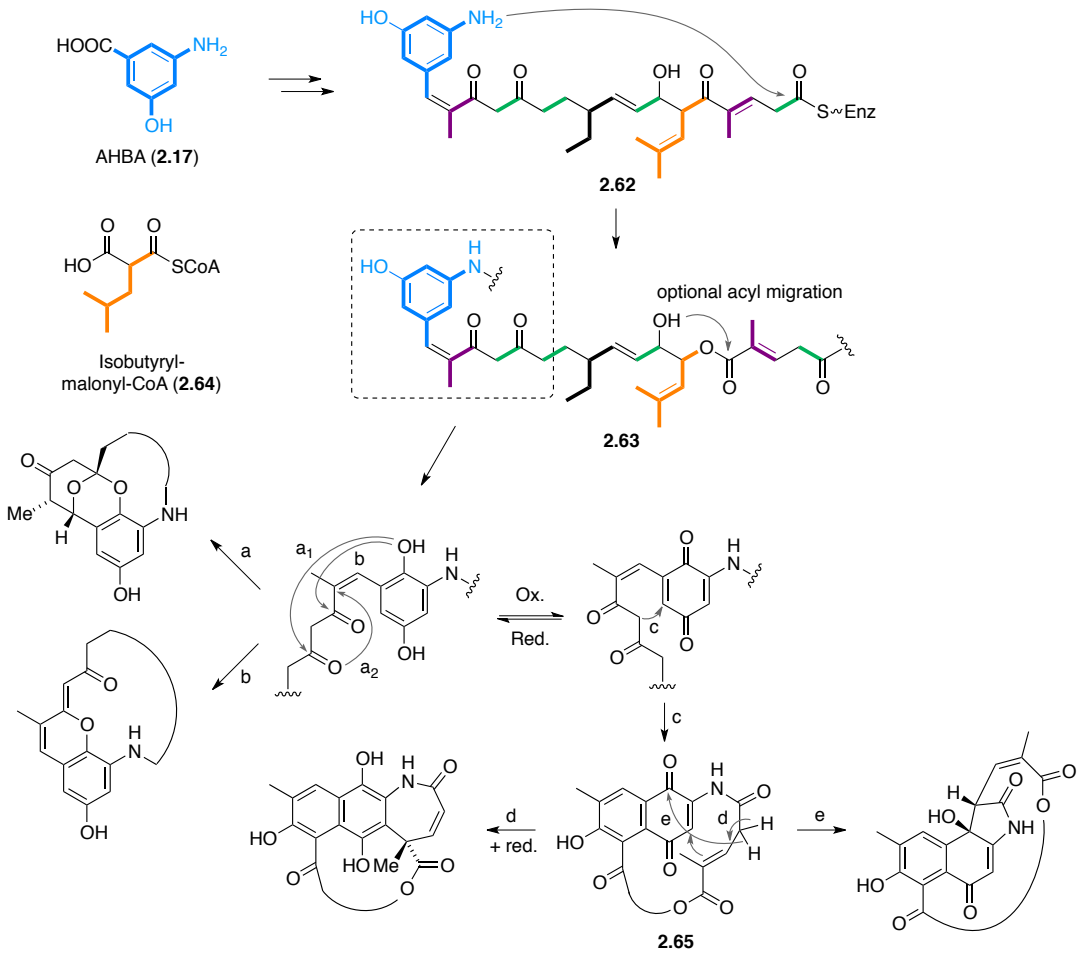
divergolide A (**2.60**) showed the strongest activity against *Mycobacterium vaccae*, while divergolide B (**2.61**) was more active against *Enterococcus faecalis*. Divergolide D (**2.4**) displayed activity against *Bacillus subtilis* and *Staphylococcus aureus* and was the only divergolide which showed pronounced activity against a number of tumor cell lines with IC_{50} values ranging from 1.0 to 2.0 μM.[31] This preliminary data renders the divergolides (particularly divergolides A and D) interesting and promising candidates for further drug development.

2.2 Biosynthesis

Although at first glance, the structures of the divergolides seem to differ profoundly, a closer inspection reveals that they are likely to originate from a common biosynthetic precursor. An intriguing biosynthesis was proposed by Hertweck *et al.* and is shown in Scheme 15. As such, the divergolides can be traced back to the AHBA-primed polyketide backbone **2.62** which is transformed into lactone intermediate **2.63** by the action of a Bayer-Villigerase.[42] The latter could undergo an optional acyl migration, that would account for the different lengths of the *ansa* chains present in the divergolides. Shifting of the double bond could occur in analogy to what has been observed for other polyketides.[43] The unsual branching in the side chain could be attributed to the incorporation of isobutyryl-malonyl-CoA (**2.64**), a novel branched extender unit.[44] The most striking feature of Hertweck`s biosynthetic proposal however is the insight that the structural diversity amongst the four divergolides could be generated through different types of cyclizations between the *ansa* chain and the aromatic moiety. Specifically, the tricyclic ring system in **2.60** would be formed *via* attack of the phenolic hydroxy group on the more distant carbonyl group to form an acetal and subsequent addition of the formed hydroxy group to the side-chain double bond (Scheme 15, route a). Alternatively, attack of the phenol at the vinylogous carbonyl group followed by elimination of water would give rise to the exomethylene-2*H*-chromene system in **2.61** (Scheme 15, route b). On the other hand, attack of the methylene group at the quinone would lead to aminonaphthoquinone intermediate **2.65** (Scheme 15, route c). This could further react *via* attack of the carbonyl-activated methylene in a Michael- (Scheme 15, route d) or Aldol-fashion (Scheme 15, route e)

[42] Gibson, M.; Nur-e-alam, M.; Lipata, F.; Oliveira, M. A.; Rohr, J. *J. Am. Chem. Soc.* **2005**, *127*, 17594–17595.
[43] (a) Taft, F.; Brünjes, T.; Knobloch, T.; Floss, H. G.; Kirschning, A. *J. Am. Chem. Soc.* **2009**, *131*, 3812–3813; (b) Moldenhauer, J.; Götz, D. C. G.; Albert, C. R.; Bischof, S. K.; Schneider, K.; Süßmuth, R.; Engeser, M.; Groß, H.; Bringmann, G.; Piel, J. *Angew. Chem. Int. Ed.* **2010**, *49*, 1465–1467; (c) Kusebauch, B.; Busch, B.; Scherlach, M.; Roth, M.; Hertweck, C. *Angew. Chem. Int. Ed.* **2010**, *49*, 1460–1464.
[44] Chan, Y. A.; Podevels, A. M.; Kevany, B. M.; Thomas, M. G. *Nat. Prod. Rep.* **2009**, *26*, 90–114.

on the quinone to give either the seven-membered lactam ring present in divergolide C (**2.3**), or the γ-lactam of divergolide D (**2.4**) (Scheme 15, route d and e).



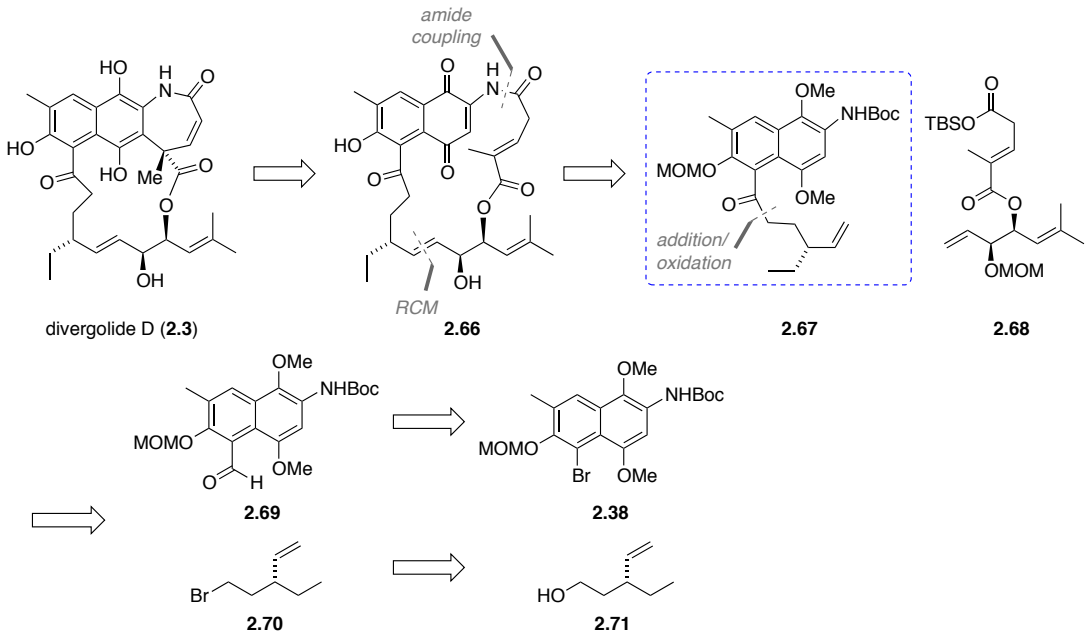
Scheme 15. Hertweck´s proposed biosynthesis of the divergolides. The different chromophores present in the divergolides could all arise from the same precursor.

2.3 Retrosynthetic Analysis

Both divergolide C (**2.3**) and divergolide D (**2.4**) share a common aminonaphtho-(hydro)quinone core, for which a synthesis had previously been developed in the course of the naphthomycin K project. This fact together with their intriguing biosynthesis and their range of biological activities motivated us to target the total synthesis of divergolides C (**2.3**) and D (**2.4**). Our approach is outlined retrosynthetically in Scheme 16, exemplified for divergolide D (**2.4**).

We envisaged that the different aromatic chromophores present in divergolides C (**2.3**) and D (**2.4**) could be formed from a common presursor by means of a biomimetic cyclization, following Hertweck´s biosynthetic proposal. Specifically, divergolide D (**2.4**) could arise from compound **2.66** by attack of the methylene group on the quinone in a vinylogous fashion and subsequent reduction. Disconnection of the macrocycle **2.66** through late stage ring-

closing metathesis and prior amide formation would lead to left-hand and right-hand fragments **2.67** and **2.68**, respectively. Naphthalene building block **2.67** could further be dissected into formyl-naphthalene **2.69** and bromide **2.70**. In a forward-sense, these fragments would be connected by lithiation of bromide **2.70** and subsequent addition into the formyl group of **2.69**, followed by oxidation. The formyl group present in **2.69** in turn was envisioned to be installed *via* lithiation of bromonapthalene **2.38** and subsequent trapping with DMF whereas bromide **2.70** could be traced back to chiral alcohol **2.71**.



Scheme 16. Retrosynthetic analysis of divergolide C (**2.3**).

The retrosynthesis of divergolide D (**2.4**) would follow the same lines with the only differences being the final formation of the heterocycle and the connectivity in fragment **2.68**.

2.4 Unpublished Results

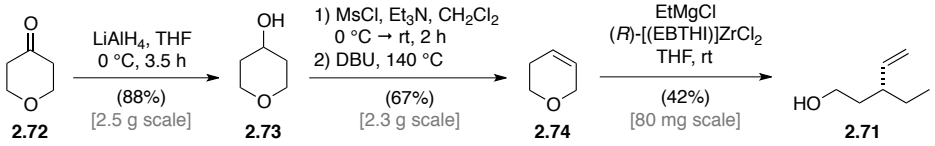
Since the synthesis of the naphthalene building block **2.38** that we planned to use in the total synthesis of divergolides C and D (**2.3** and **2.4**) is already described in section 1.3.2.2, the following section will only cover results towards an enantioselective synthesis of building block **2.71**.

At the outset of our studies, we found two publications reporting the enantioselective synthesis of (*R*)-alcohol **2.71**. While we considered Liang´s route[45] to **2.71** as less suitable for our purposes, the one-step protocol published by Morken *et al.*[46] seemed more attractive.

[45] Liang, B.; Negishi, E. *Org. Lett.* **2008**, *10*, 193–195.
[46] Morken, J. P.; Didiuk, M. T.; Hoveyda, A. H. *J. Am. Chem. Soc.* **1993**, *115*, 6997–6998.

Accordingly, the requisite pyran **2.74** was prepared in three steps from commercially available pyranone **2.72** as depicted in Scheme 17. Thus, treatment of ketone **2.72** with $LiAlH_4$ in THF furnished the corresponding alcohol **2.73** in 88% yield. Subsequent mesylation followed by DBU-mediated dehydration afforded pyran **2.74**.[47]

Compound **2.74** was next subjected to zirconium-catalyzed asymmetric carbomagnesation following the literature procedure to yield alcohol **2.71** in 42% yield.[37]



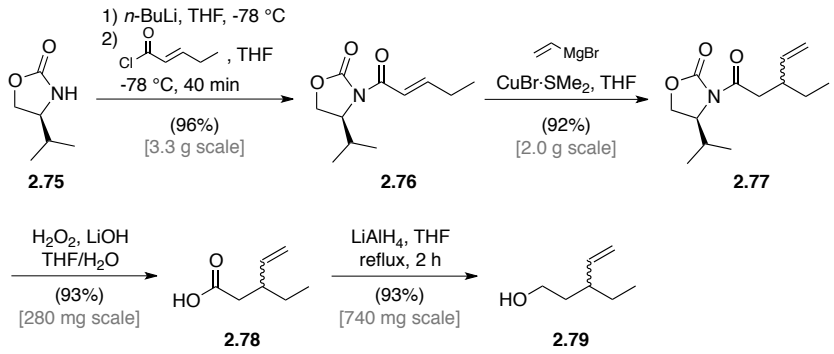
Scheme 17. Four-step synthesis of **2.71** using an enantioselective carbomagnesation reaction.

Although this route is quite short, it was abandoned since it requires the use of an expensive catalyst and is thus not readily scalable.

We next aimed for a more practical route towards alcohol **2.71,** starting from inexpensive materials. For comparison purposes, Morken *et al.* had synthesized the enantiomer of alcohol **2.71** using an asymmetric conjugate addition approach. A similar approach was next attempted, albeit starting from chiral oxazolidinone auxiliary **2.75**, which was readily available from valine.

The planned synthesis of **2.71** commenced with deprotonation of (*S*)-4-isopropyloxazolidin-2-one (**2.75**) with *n*-BuLi, followed by acylation with freshly prepared (*E*)-pent-2-enoyl chloride[48] to give imide **2.76** in excellent yield (Scheme 18). Conjugate addition of vinylmagnesium bromide in the presence of $CuBr{\cdot}SMe_2$ in THF afforded alkene **2.77** in very good yield, albeit as a 2:1 mixture of diastereoisomers that could not be separated by flash column chromatography. Although it was already clear at this point that the diastereoselectivity of this reaction was not acceptably high to merit adopting this route, the material in hand was used to investigate the subsequent steps. Thus, reaction of alkene **2.77** with lithium hydroperoxide in THF/H_2O effected the removal of the chiral auxiliary and gave acid **2.78** in very good yield.[49] Finally, reduction to the corresponding, volatile alcohol **2.79** was achieved in 93% by refluxing the acid **2.78** with $LiAlH_4$ in THF.

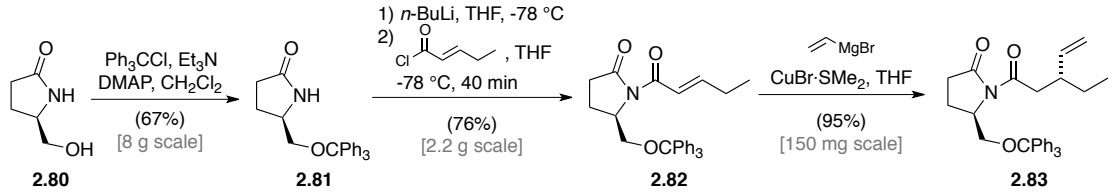
[47] Suto, M. J.; Stier, M. A.; Werbel, L. M.; Arundel-Suto, C. M.; Leopold, W. R.; Elliott, W. E.; Sebolt-Leopold, J. S. *J. Med. Chem.* **1991**, *34*, 2484–2488.
[48] Zhang, F.-L.; Schweizer, W. B.; Xu, M.; Vasella, A. *Helv. Chim. Acta* **2007**, *90*, 521–534.
[49] Zask, A.; Birnberg G.; Cheung, K.; Kaplan, J.; Niu, C.; Norton, E.; Suayan, R.; Yamashita, A.; Cole, D.; Tang, Z.; Krishnamurthy, G.; Williamson, R.; Khafizova, G.; Musto, S.; Hernandez, R.; Annable, T.; Yang, X.;



Scheme 18. Using auxiliary **2.75**, the desired alcohol **2.71** could be synthesized, albeit as a mixture of enantiomers.

In spite of the fact that this alternative route was short, robust and scalable, it was not suitable because of the poor asymmetric induction from chiral auxiliary **2.75**.

Based on the alternative route that Morken *et al.* had used to synthesize the (*S*)-enantiomer of **2.71** using (*S*)-γ-trityloxymethyl-γ-butyrolactam[50] as a chiral auxiliary, we reasoned that its enantiomer (*R*)-γ-trityloxymethyl-γ-butyrolactam (**2.81**) should give rise to the desired (*R*)-configured alcohol **2.71**. The preparation of chiral auxiliary **2.81** started with tritylation[51] of literature known lactam alcohol **2.80**[52] in 67% yield (Scheme 19). The resulting lactam ether **2.81** was next acylated to the imide **2.82** in 76% yield as described above by following a procedure from Evans *et al.*[53] The following asymmetric conjugate addition was carried out as described by Tomioka *et al.*[41] and afforded the α,β-unsaturated imide **2.83** as a single diastereoisomer. Due to time constraints, I had to leave the project at this point. However, it could subsequently been shown by Anastasia Hager, a Ph.D. student in our group, that this route furnished the desired enantiopure (*R*)-alcohol **2.71**.



Scheme 19. Use of (*R*)-γ-trityloxymethyl-γ-butyrolactam (**2.81**) allowed for a diastereoselective synthesis of intermediate **2.83**.

Discafani, C.; Beyer, C.; Greenberger, L. M.; Loganzo, F.; Ayral-Kaloustian, S. *J. Med. Chem.* **2004**, *47*, 4774–4786.

[50] Tomioka, K.; Suenaga, T.; Koga, J. *Tetrahedron Lett.* **1986**, *27*, 369–372.

[51] Adam, W.; Zhang, A. *Eur. J. Org. Chem.* **2004**, 147–152.

[52] (a) Amstutz, R.; Ringdahl, B.; Karlen, B.; Roch, M.; Jenden, D. J. *J. Med. Chem.* **1985**, *28*, 1760–1765; (b) Valasinas, A.; Frydman, B.; Friedmann, H. C. *J. Org. Chem.* **1992**, *57*, 2158–2160.

[53] Evans, D. A.; Bartroli, J.; Shih, T. L. *J. Am. Chem. Soc.* **1981**, *103*, 2127–2129.

3 Experimental Part

3.1 General Experimental Details

Unless stated otherwise, all reactions were performed in oven-dried or flame-dried glassware under a positive pressure of nitrogen. Commercial reagents and solvents were used as received with the following exceptions. Tetrahydrofuran (THF) was distilled from benzophenone and sodium immediately prior to use. Triethylamine, diisopropylamine and diisopropylethylamine were distilled over calcium hydride immediately before use. Reactions were magnetically stirred and monitored by NMR spectroscopy or analytical thin-layer chromatography (TLC) using E. Merck 0.25 mm silica gel 60 F_{254} precoated glass plates. TLC plates were visualized by exposure to ultraviolet light (UV, 254 nm) and/or exposure to an aqueous solution of ceric ammoniummolybdate (CAM), an aqueous solution of potassium permanganate ($KMnO_4$), an acidic solution of vanillin or a solution of ninhydrin in ethanol followed by heating with a heat gun. Flash column chromatography was performed as described by Still *et al.* employing silica gel (60 Å, 40-63 μm, Merck) and a forced flow of eluant at 1.3–1.5 bar pressure.[54] Yields refer to chromatographically and spectroscopically (^{1}H and ^{13}C NMR) pure material.

3.2 Instrumentation

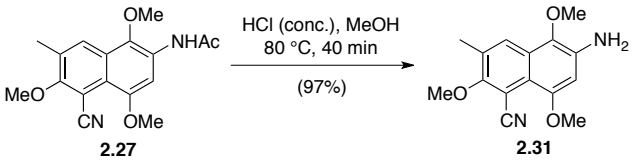
Proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded on Varian VNMRS 300, VNMRS 400, INOVA 400 or VNMRS 600 spectrometers. Proton chemical shifts are expressed in parts per million (δ scale) and are calibrated using residual undeuterated solvent as an internal reference ($CDCl_3$: δ 7.26, CD_2Cl_2: δ 5.32, DMSO-d_6: δ 2.50). Data for ^{1}H NMR spectra are reported as follows: chemical shift (δ ppm) (multiplicity, coupling constant (Hz), integration). Multiplicities are reported as follows: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, *br* = broad, app = apparent, or combinations thereof. Carbon nuclear magnetic resonance (^{13}C NMR) spectra were recorded on Varian VNMRS 300, VNMRS 400, INOVA 400 or VNMRS 600 spectrometers. Carbon chemical shifts are expressed in parts per million (δ scale) and are referenced to the carbon resonances of the solvent ($CDCl_3$: δ 77.0, CD_2Cl_2: δ 53.84, DMSO-d_6: δ 39.5). Infrared (IR) spectra were recorded on a Perkin Elmer Spectrum BX II (FTIR System). IR data is reported in frequency of absorption (cm^{-1}). Mass spectroscopy (MS) experiments were performed on a Thermo Finnigan MAT 95 (EI) or on a

[54] Still, W. C.; Kahn, M.; Mitra, A. *J. Org. Chem.* **1978**, *43*, 2923–2925.

Thermo Finnigan LTQ FT (ESI) instrument. Optical rotations were obtained on a Perkin Elmer 241 Polarimeter.

3.3 Synthetic Procedures

3.3.1 Naphthomycin K Project



Amine 2.31:

To a suspension of cyano-naphthalene **2.27** (67 mg, 213 µmol, 1.0 equiv.) in MeOH (4 mL) in a pressure tube was added conc. HCl (0.5 mL). The pressure tube was sealed and heated to 80 °C in an oil bath for 40 min. After cooling to rt, the reaction mixture was adjusted to pH = 7 by addition of an aq. solution of NaOH (2 M). The resulting white suspension was extracted with $CHCl_3$ (3 × 10 mL) and the combined organic layers were, dried over sodium sulfate, filtered and concentrated under reduced pressure. Purification of the residue by flash column chromatography (silica gel, $CHCl_3$:acetone = 25:1) provided amine **2.31** (56 mg, 206 µmol, 97%) as a grey solid.

TLC ($CHCl_3$:acetone = 10:1), R_f = 0. 4 (UV/CAM)

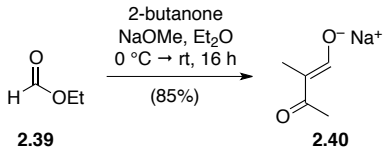
M.p.: 110–115 °C

^{1}H NMR (600 MHz, $CDCl_3$) δ: 7.84–7.84 (m, 1 H), 6.44 (s, 1 H), 4.00 (s, 3 H), 3.96 (s, 3 H), 3.80 (s, 3 H), 2.44 (d, J = 0.8 Hz, 3 H).

^{13}C NMR (150 MHz, $CDCl_3$) δ: 161.2, 151.5, 136.0, 133.0, 131.8, 126.6, 126.5, 117.6, 116.9, 99.7, 99.5, 61.6, 60.1, 55.8, 16.8.

IR (Diamond-ATR, neat) ν_{max}: 2359, 2338, 2210, 2156, 1956, 1732, 1716, 1699, 1683, 1652, 1622, 1558, 1539, 1505, 1472, 1456, 1388, 1236, 1201, 1064, 1001, 963, 899, 833, 812, 668 cm^{-1}.

HRMS (ESI) calcd for $C_{15}H_{17}N_2O_3$ $[M+H]^+$: 273.1234; found: 273.1235.

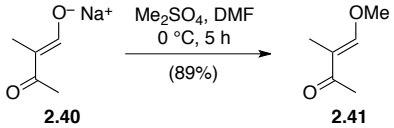


2-methyl-3-oxobutanal sodium salt (2.40):

To a suspension of sodium methoxide (10.4 g, 192 mmol, 0.96 equiv.) in dry Et_2O (180 mL) was added a mixture of 2-butanone (17.9 mL, 200 mmol, 1.00 equiv.) and ethyl formate (**2.39**) (17.8 mL, 240 mmol, 1.20 equiv.) over 5 min at 0 °C. The reaction mixture was warmed to rt and stirred for 16 h. The white precipitate was filtered off, washed with Et_2O (2 × 100 mL) and dried under high vacuum overnight to yield the product **2.40** (20.7 g, 170 mmol, 85%) as a white solid.

^{1}H NMR (400 MHz, D_2O) δ: 9.01 (s, 1 H), 2.21 (s, 3 H), 1.60 (s, 3 H).

HRMS (EI) calcd for $C_5H_7O_2Na$ $[M]^{+\bullet}$: 122.0344; found: 122.0342.



(*E*)-4-methoxy-3-methylbut-3-en-2-one (2.41):

2.40 (10.0 g, 82 mmol, 1.0 equiv.) was dissolved in dry DMF (100 mL) and the solution was cooled to 0 °C. Dimethyl sulfate (15.5 mL, 164 mmol, 2.0 equiv.) was added over 30 min via syringe pump and the reaction mixture was stirred at 0 °C for 5 h. An aq. solution of K_2CO_3 (1 M, 100 mL) and MeOH (6 mL) were added and the reaction mixture was warmed to rt. The mixture was extensively extracted with EtOAc until TLC monitoring showed that the extraction was complete. The combined organic layers were washed with an aq. solution of LiCl (10%, 500 mL) (if TLC indicated product in the aq. LiCl solution layer, it was re-extracted with EtOAc), dried over sodium sulfate and concentrated *in vacuo*. The crude material was purified by flash column chromatography (silica gel, gradient: hexanes:Et_2O = 2:1 → 1:1) to afford the title compound **2.41** (8.32 g, 72.9 mmol, 89%) as a light yellow oil.

Note: Due to its volatility, the pressure should not be lower than 100 mbar (at 40 °C water bath temperature) during rotary evaporation. The clean product should also be stored at -78 °C due to its tendency to decompose.

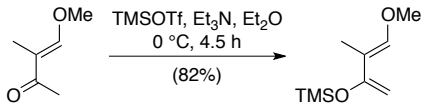
TLC (hexanes:EtOAc = 1:1), R_f = 0.41 ($KMnO_4$)

^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.19 (q, J = 1.2 Hz, 1 H), 3.83 (s, 3 H), 2.16 (s, 3 H), 1.65 (d, J = 1.2 Hz, 3 H).

^{13}C-NMR (75 MHz, $CDCl_3$) δ = 197.3, 160.2, 117.6, 61.2, 25.1, 8.0.

IR (Diamond-ATR, neat) ν_{max}: 2942, 1631, 1455, 1396, 1368, 1303, 1239, 1143, 1109, 973, 935, 823, 787, 755, 732 cm^{-1}.

HRMS (EI) calcd for $C_6H_{10}O_2$ $[M]^{+\bullet}$: 114.0681; found: 114.0677.



2.41 **2.42**

(*E*)-((4-methoxy-3-methylbuta-1,3-dien-2-yl)oxy)trimethylsilane (2.42)

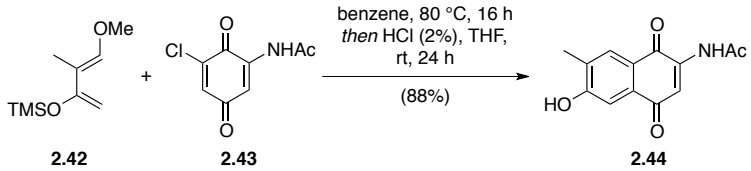
To a solution of enol ether **2.41** (2.76 g, 24.2 mmol, 1.0 equiv.) in dry Et_2O (70 mL) was added triethylamine (8.4 mL, 60.5 mmol, 2.5 equiv.) at rt. The cloudy solution was stirred for 5 min, cooled to 0 °C and a solution of TMSOTf (5.25 mL, 29.0 mmol, 1.2 equiv.) in dry Et_2O (10 mL) was added dropwise. The brown-orange reaction mixture was stirred at 0 °C for 4.5 h and subsequently, hexanes (30 mL) and sat. aq. $NaHCO_3$ (50 mL) were added. The organic layer was separated and the aq. layer was extracted with hexanes (3 × 100 mL). The combined organic layers were washed with water (1 × 200 mL) and sat. aq. NaCl (1 × 300 mL), dried over sodium sulfate, filtered and subjected to rotary evaporation (100 mbar). The residue was purified by fractional distillation (42 °C, $9 \cdot 10^{-1}$ mbar) to afford the product **2.42** (3.68 g, 19.8 mmol, 82%) as a colorless oil.

^{1}H NMR (300 MHz, $CDCl_3$) δ: 6.51–6.50 (m, 1 H), 4.23 (d, *J* = 1.4 Hz, 1 H), 4.12 (d, *J* = 1.3 Hz, 1 H), 3.67 (s, 3 H), 1.69 (d, *J* = 1.3 Hz, 3 H), 0.23 (s, 9 H).

^{13}C-NMR (75 MHz, $CDCl_3$) δ = 156.2, 147.1, 111.4, 89.1, 60.1, 10.0, 0.1.

IR (Diamond-ATR, neat) ν_{max}: 2958, 2840, 2361, 2340, 1656, 1590, 1456, 1387, 1353, 1306, 1252, 1228, 1136, 1019, 1005, 972, 840, 816, 753, 689 cm^{-1}.

HRMS (EI) calcd for $C_9H_{18}O_2Si$ $[M]^{+\bullet}$: 186.1076; found: 186.1063.



2.42 **2.43** **2.44**

Acetyl-aminonaphthoquinone 2.44:

A pressure tube was charged with quinone **2.43** (3.37 g, 16.9 mmol, 1.0 equiv.) and dry benzene (60 mL). To this suspension was added a solution of diene **2.42** (3.33 g, 17.7 mmol, 1.05 equiv.) in dry benzene (15 mL). The pressure tube was sealed and the orange reaction mixture was heated to 80 °C for 16 h. The mixture was cooled to rt, transferred into a round bottom flask and the solvent was removed *in vacuo*. The residue was dissolved in THF (100 mL) and HCl (2%, 20 mL) was added. The resulting solution turned black and was stirred under ambient atmosphere at rt for 24 h. Silica gel was added, the reaction mixture was concentrated, and the crude was loaded directly onto a silica gel column for chromatography (gradient: $CHCl_3$:acetone = 20:1 → 10:1 → 5:1 → 1:1; then CH_2Cl_2:MeOH = 5:1 → 2:1). The fractions containing the product were combined and filtered and the eluent was removed under reduced pressure to afford acetyl-aminonaphthoquinone **2.44** (3.64 g, 14.8 mmol, 88%) as a crystalline, orange-brown powder.

TLC ($CHCl_3$:acetone = 10:1), R_f = 0.56 (visible/CAM)

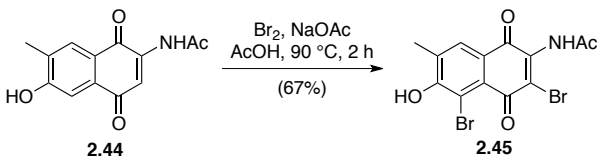
M.p.: 220 °C (decomp.)

^{1}H NMR (400 MHz, DMSO-d_6) δ: 11.05 (s, 1 H), 9.80 (s, 1 H), 7.79 (d, J = 0.8 Hz, 1 H), 7.55 (s, 1 H), 7.30 (s, 1 H), 2.23 (s, 3 H), 2.22 (s, 3 H).

^{13}C NMR (100 MHz, DMSO-d_6) δ: 185.3, 179.2, 171.2, 161.7, 141.4, 131.8, 130.3, 129.6, 121.8, 115.4, 110.5, 24.6, 15.9.

IR (Diamond-ATR, neat) ν_{max}: 3338, 1721, 1653, 1629, 1609, 1573, 1483, 1336, 1317, 1260, 1196, 1066, 1000, 964, 876, 788, 739, 695 cm^{-1}.

HRMS (EI) calcd for $C_{13}H_{11}NO_4$ $[M]^{+\bullet}$: 245.0688; found: 245.0684.



2.44 **2.45**

Dibromo-naphthoquinone 2.45

To a suspension of acetyl-aminonaphthoquinone **2.44** (40 mg, 0.16 mmol, 1.0 equiv.) and NaOAc (44 mg, 0.54 mmol, 3.3 equiv.) in conc. AcOH (3 mL) was added a solution of

bromine (9 µL, 0.17 mmol, 1.05 equiv.) in conc. AcOH (1 mL) at rt. The reaction mixture was heated at 90 °C for 2 h and then cooled to rt. Water (5 mL) was added and the mixture was extracted with $CHCl_3$ (3 × 10 mL). The combined organic layers were dried over sodium sulfate, filtered and concentrated *in vacuo*. Purification of the crude product by flash column chromatography (silica gel, gradient: $CHCl_3$:acetone = 15:1 → 10:1) afforded the product **2.45** (44 mg, 109 µmol, 67%) as a yellow solid.

TLC ($CHCl_3$:acetone = 10:1), R_f = 0.38 (UV/CAM)

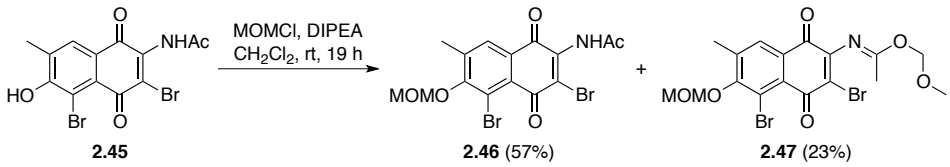
M.p.: 197 °C

^{1}H NMR (400 MHz, DMSO-d_6) δ: 10.00 (s, 1 H), 7.83 (d, J = 0.8 Hz, 1 H), 2.37 (d, J = 0.7 Hz, 3 H), 2.09 (s, 3 H).

^{13}C-NMR (100 MHz, DMSO-d_6) δ = 176.8, 176.5, 167.9, 158.3, 142.9, 131.8, 130.7, 129.1, 127.9, 125.0, 110.9, 22.9, 17.4.

IR (Diamond-ATR, neat) ν_{max}: 3241, 2361, 1686, 1664, 1649, 1619, 1579, 1540, 1502, 1470, 1415, 1372, 1342, 1256, 1217, 1171, 1085, 1042, 1020, 982, 917, 859, 831, 820, 758, 706, 679, 664 cm^{-1}.

HRMS (ESI) calcd for $C_{13}H_8Br_2NO_4$ $[M-H]^-$: 399.8826; found: 399.8826.



2.45 **2.46** (57%) **2.47** (23%)

MOM-Dibromo-naphthoquinones 2.46 and **2.47**

To a solution of **2.45** (49 mg, 122 µmol, 1.0 equiv.) in dry CH_2Cl_2 (6 mL) was added DIPEA (53 µL, 302 µmol, 2.0 equiv.) and at rt. The solution was cooled to 0 °C, MOMCl (37 µL, 435 µmol, 3.6 equiv.) was added and the reaction mixture was warmed to rt and stirred for 19 h before it was quenched by addition of sat. aq. $NaHCO_3$ (5 mL). The mixture was extracted with $CHCl_3$ (3 × 10 mL) and the combined org. layers were dried over sodium sulfate, filtered and concentrated under reduced pressure. The residue was purified by flash column chromatography (silica gel, gradient: $CHCl_3$:acetone = 30:1) to yield **2.46** (31 mg, 69 µmol, 57%) and **2.47** (14 mg, 28 µmol, 23%) as a yellow solid.

*Analytical data for compound **2.46**:*

TLC (hexanes:EtOAc = 10:1), R_f = 0.30 (UV/CAM)

M.p.: 194 °C

^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.96 (d, *J* = 0.7 Hz, 1 H), 7.58 (*br* s, 1 H), 5.17 (s, 2 H), 3.66 (s, 3 H), 2.50 (d, *J* = 0.7 Hz, 3 H), 2.27 (s, 3 H).

^{13}C-NMR (100 MHz, $CDCl_3$) δ = 177.8, 175.8, 166.6, 160.3, 141.3, 139.7, 130.0, 129.3, 128.7, 128.6, 119.6, 100.4, 58.1, 24.2, 18.1.

IR (Diamond-ATR, neat) ν_{max}: 3243, 1684, 1668, 1656, 1612, 1580, 1519, 1457, 1374, 1323, 1283, 1260, 1224, 1189, 1168, 1085, 1036, 980, 921, 858, 835, 758, 734, 706, 692 cm^{-1}.

HRMS (ESI) calcd for $C_{15}H_{14}Br_2NO_5$ $[M+H]^+$: 445.9233; found: 445.9239.

*Analytical data for compound **2.47**:*

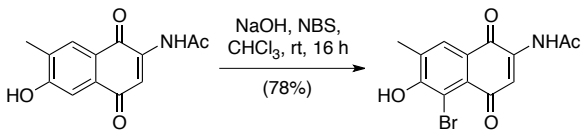
TLC ($CHCl_3$:acetone = 10:1), R_f = 0.87 (UV/CAM)

M.p.: 183 °C

^{1}H NMR (600 MHz, $CDCl_3$) δ: 7.96 (s, 1 H), 5.46 (s, 2 H), 5.18 (s, 2 H), 3.67 (s, 3 H), 3.59 (s, 3 H), 2.50 (s, 3 H), 1.92 (s, 3 H).

^{13}C-NMR (150 MHz, $CDCl_3$) δ = 177.2, 176.5, 162.3, 159.9, 149.7, 139.1, 130.0, 129.2, 128.6, 123.7, 119.1, 100.4, 93.7, 58.1, 58.0, 18.8, 18.0.

IR (Diamond-ATR, neat) ν_{max}: 3242, 1684, 1667, 1611, 1581, 1521, 1376, 1322, 1262, 1224, 1206, 1170, 1158, 1082, 1020, 980, 922, 906, 846, 759, 737, 691 cm^{-1}.



2.44 **2.48**

Bromo-aminonaphthoquinone 2.48:

To a suspension of Acetyl-protected aminonaphthoquinone **2.44** (100 mg, 0.41 mmol, 1.0 equiv.) in $CHCl_3$ (50 mL) was added NaOH (20 mg, 0.49 mmol, 1.2 equiv.) and *N*-Bromosuccinimide (87 mg, 0.49 mmol, 1.2 equiv.) and the orange reaction mixture was stirred at rt for 16 h. The solvent was then evaporated *in vacuo* and the crude product was purified by flash column chromatography (silica gel, $CHCl_3$:acetone = 20:1) to provide the title compound **2.48** (103 mg, 0.32 mmol, 78%) as an orange solid.

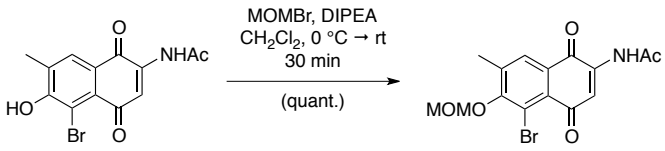
TLC ($CHCl_3$:acetone = 10:1), R_f = 0.49 (visible/UV/CAM)

M.p.: 183 °C (decomp.)

^{1}H NMR (400 MHz, DMSO-d_6) δ: 10.40 (*br* s, 1 H), 9.82 (s, 1 H), 7.89 (s, 1 H), 7.57 (s, 1 H), 2.36 (s, 3 H), 2.21 (s, 3 H).

^{13}C NMR (150 MHz, DMSO-d_6) δ: 193.3, 184.0, 178.9, 171.2, 158.7, 139.8, 130.8, 129.0, 128.0, 124.4, 117.3, 24.4, 17.3.

HRMS (ESI) calcd for $C_{13}H_{10}BrNO_4$ $[M-H]^-$: 321.9720; found: 321.9723.



2.48 **2.49**

Aminonaphthoquinone 2.49:

Bromo-naphthoquinone **2.48** (103 mg, 0.32 mmol, 1.0 equiv.) was dissolved in dry CH_2Cl_2 (15 mL) and the clear yellow solution was cooled to 0 °C. DIPEA (111 µL, 0.64 mmol, 2.0 equiv.) was added dropwise, upon which the solution rapidly turned purple. Bromomethyl methyl ether (34 µL, 0.41 mmol, 1.3 equiv.) was added dropwise and the resulting yellow solution was warmed to rt and stirred for 30 min. Sat. aq. $NaHCO_3$ (10 mL) was then added and the layers separated. The aqueous layer was extracted with $CHCl_3$ (3 × 15 mL), the combined organics were dried over sodium sulfate, filtered and the solvent was removed under reduced pressure. Purification of the residue by flash column chromatography (silica gel, $CHCl_3$:acetone = 20:1) afforded MOM-ether **2.49** (117 mg, 0.32 mmol, 100%) as a yellow solid.

TLC ($CHCl_3$:acetone = 10:1), R_f = 0.73 (UV/CAM)

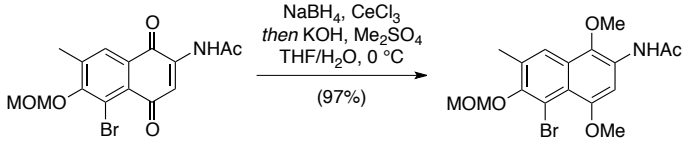
M.p.: 117 °C

^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.22 (*br* s, 1 H), 8.00 (d, J = 0.7 Hz, 1 H), 7.80 (s, 1 H), 5.18 (s, 2 H), 3.67 (s, 3 H), 2.50 (d, J = 0.7 Hz, 3 H), 2.27 (s, 3 H).

^{13}C NMR (75 MHz, $CDCl_3$) δ: 183.6, 179.9, 169.2, 160.6, 138.6, 138.2, 129.8, 129.1, 128.2, 119.0, 118.1, 100.4, 58.1, 25.0, 17.9.

IR (Diamond-ATR, neat) ν_{max}: 3292, 3100, 2924, 1719, 1671, 1638, 1585, 1528, 1338, 1276, 1257, 1226, 1158, 1076, 1020, 971, 912, 798, 750, 732 cm^{-1}.

HRMS (ESI) calcd for $C_{15}H_{14}BrNO_5$ $[M+H]^+$: 368.0128; found: 368.0131.



2.49 **2.37**

Hydroquinone dimethyl ether 2.37:

MOM-ether **2.49** (200 mg, 543 µmol, 1.0 equiv.) was dissolved in a 2:1 mixture of THF (20 mL) and water (10 mL) and Cerium(III)-chloride heptahydrate (304 mg, 0.81 mmol, 1.50 equiv.) was added. The reaction mixture was cooled to 0 °C and $NaBH_4$ (41 mg, 1.09 mmol, 2.0 equiv.) was added in two portions over 5 min. After 15 min, a solution of KOH (610 mg, 10.9 mmol, 20 equiv.) in H_2O (13 mL) was added to the clear solution. The resulting brown reaction mixture was stirred for 15 min and Me_2SO_4 (1.55 mL, 16.3 mmol, 30 equiv.) was next added dropwise. After 2 h, conc. NH_4OH (10 mL) and H_2O (20 mL) were added, the reaction mixture was warmed to rt and extracted with EtOAc (3 × 50 mL) and $CHCl_3$ (3 × 20 mL). The combined organic layers were dried over sodium sulfate, filtered and concentrated under reduced pressure. Purification of the residue by flash column chromatography (silica gel, $CHCl_3$:acetone = 20:1) afforded the title compound **2.37** (209 mg, 0.52 mmol, 97%) as a beige solid.

TLC ($CHCl_3$:acetone = 10:1), R_f = 0.5 (UV/CAM)

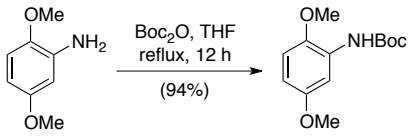
M.p.: 168 °C

^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.06 (s, 1 H), 7.78 (*br* s, 1 H), 7.72 (s, 1 H), 5.16 (s, 2 H), 3.95 (s, 3 H), 3.83 (s, 3 H), 3.68 (s, 3 H), 2.53 (d, *J* = 0.9 Hz, 3 H), 2.27 (s, 3 H).

^{13}C NMR (75 MHz, $CDCl_3$) δ: 168.5, 152.8, 152.4, 135.6, 133.1, 127.8, 127.1, 122.2, 120.8, 111.9, 101.5, 100.1, 61.5, 57.8, 56.0, 25.0, 18.4.

IR (Diamond-ATR, neat) ν_{max}: 3233, 2947, 1662, 1621, 1607, 1572, 1529, 1458, 1401, 1369, 1319, 1272, 1241, 1224, 1150, 1099, 1073, 1037, 998, 981, 962, 919, 878, 827, 786, 759, 733, 702, 677 cm^{-1}.

HRMS (ESI) calcd for $C_{17}H_{20}BrNO_5$ $[M+H]^+$: 398.0603; found: 398.0602.



2.50 **2.51**

***tert*-Butyl (2,5-dimethoxyphenyl)carbamate (2.51)**

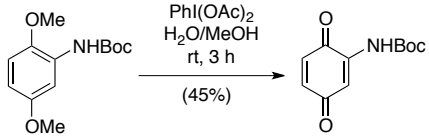
To a solution of 2,5-dimethoxyaniline (**2.50**) (20 g, 163 mmol, 1.0 equiv.) in dry THF (100 mL) was added Boc_2O (34.2 g, 196 mmol, 1.2 equiv.) and the black reaction mixture was heated under reflux for 12 h. After cooling to rt, the solvent was evaporated *in vacuo* and the crude product was purified by flash column chromatography (silica gel, gradient: hexanes:EtOAc = 10:1 → 5:1) to afford the product **2.51** (38.6 g, 153 mmol, 94%) as a viscous light yellow oil.

TLC (hexanes:EtOAc = 5:1), R_f = 0.38 (UV/CAM)

^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.79 (s, 1 H), 7.09 (s, 1 H), 6.76 (d, *J* = 8.9 Hz, 1 H), 6.49 (dd, *J* = 8.9, 3.0 Hz, 1 H), 3.82 (s, 3 H), 3.78 (s, 3 H), 1.53 (s, 9 H).

^{13}C-NMR (75 MHz, $CDCl_3$) δ = 154.1, 152.6, 141.7, 128.9, 110.8, 107.1, 104.3, 80.3, 56.2, 55.8, 28.4.

HRMS (EI) calcd for $C_{13}H_{19}NO_4$ $[M]^{+\bullet}$: 253.1314; found: 253.1304.



2.51 **2.52**

2-*tert*-Butoxycarbonylamino-1,4-benzoquinone (2.52)

To a solution of **2.51** (1.0 g, 3.95 mmol, 1.0 equiv.) in a mixture of water (20 mL) and MeOH (0.5 mL) was added $PhI(OAc)_2$ (3.18 g, 5.93 mmol, 2.5 equiv.) and the resulting suspension was stirred for 3 h at rt. Water (60 mL) was added and the mixture was extracted with CH_2Cl_2 (3 × 40 mL). The combined organic layers were washed with water (120 mL), dried over sodium sulfate, filtered and concentrated *in vacuo*. The resulting dark-brown residue was purified by flash column chromatography (silica gel, gradient: hexanes:EtOAc = 20:1 → 15:1) to afford the product **2.52** (393 mg, 1.76 mmol, 45%) as a yellow solid

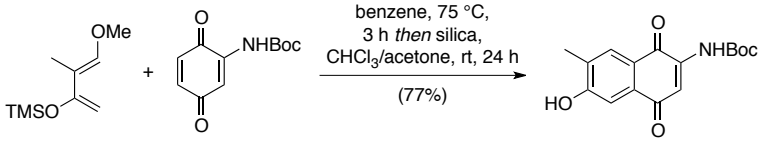
Note: When the reaction was scaled up to 20 g, the yield dropped to 19%.

TLC (hexanes:EtOAc = 1:1), R_f = 0.77 (UV/CAM)

^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.38 (*br* s, 1 H), 7.21 (d, *J* = 2.2 Hz, 1 H), 6.73 (s, 1 H), 6.72 (d, *J* = 2.1 Hz, 1 H), 1.51 (s, 9 H).

^{13}C-NMR (75 MHz, $CDCl_3$) δ = 187.4, 182.4, 151.0, 139.3, 138.2, 133.1, 112.5, 82.7, 28.1.

HRMS (EI) calcd for $C_{11}H_{13}NO$ $[M]^{+\bullet}$: 223.0845; found: 223.0837.



2.42 2.52 2.53

Boc-aminonaphthoquinone 2.53:

A pressure tube was charged with diene **2.42** (1.31 g, 7.0 mmol, 1.0 equiv), Boc-quinone **2.52** (1.56 g, 7.0 mmol, 1.0 equiv.) and dry benzene (10 mL), sealed and heated to 75 °C for 3 h. The yellow reaction mixture was cooled to rt and washed with HCl (1 M, 10 mL). The organic layer was separated, dried over sodium sulfate, filtered and the solvent was removed under reduced pressure. The crude residue was dissolved in a 1:1 mixture of $CHCl_3$ (25 mL) and acetone (25 mL) and oven-dried silica gel (5 g) was added. The suspension was stirred under ambient atmosphere for 24 h and subsequently concentrated under reduced pressure. The crude material was purified by flash column chromatography (silica gel, dry load, gradient: $CHCl_3$:acetone = 30:1 → 10:1 → 5:1 → 2:1 → 1:1) to afford the product **2.53** (1.48 g, 4.88 mmol, 77%) as an orange solid.

TLC ($CHCl_3$:acetone = 20:1), R_f = 0.26 (visible/CAM)

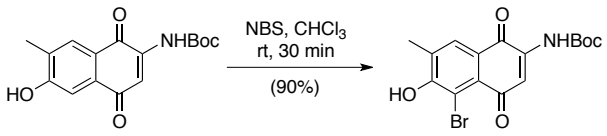
M.p.: 320 °C (decomp.)

^{1}H NMR (400 MHz, DMSO-d_6) δ: 11.07 (s, 1 H), 8.51 (s, 1 H), 7.77 (d, J = 0.8 Hz, 1 H), 7.30 (s, 1 H), 7.11 (s, 3 H), 2.22 (d, J = 0.5 Hz, 3 H), 1.48 (s, 9 H).

^{13}C NMR (100 MHz, DMSO-d_6) δ: 184.4, 178.5, 161.8, 151.5, 141.8, 131.9, 130.2, 129.6, 121.7, 113.3, 110.7, 81.7, 27.7, 15.9.

IR (Diamond-ATR, neat) ν_{max}: 3326, 1737, 1664, 1632, 1609, 1578, 1496, 1335, 1225, 1202, 1143, 1079, 1042, 1014, 962, 915, 876, 810, 802, 768, 736 cm^{-1}.

HRMS (ESI) calcd for $C_{16}H_{17}NO_5$ $[M+H]^+$: 304.1179; found: 304.1181.



2.53 **2.54**

Bromo-aminonaphthoquinone 2.54:

To a solution of Boc-protected aminonaphthoquinone **2.53** (1.20 g, 3.94 mmol, 1.0 equiv.) in $CHCl_3$ (300 mL) was added *N*-Bromosuccinimide (841 mg, 4.72 mmol, 1.2 equiv.) and the orange reaction mixture was stirred at rt for 30 min. The solvent was evaporated *in vacuo* and the crude product was purified by flash column chromatography (silica gel packed in $CHCl_3$, gradient: hexanes:EtOAc = 10:1 → 5:1 → 2:1) to provide the target compound **2.54** (1.36 g, 3.56 mmol, 90%) as a yellow solid.

TLC ($CHCl_3$:acetone = 10:1), R_f = 0.78 (visible/UV/CAM)

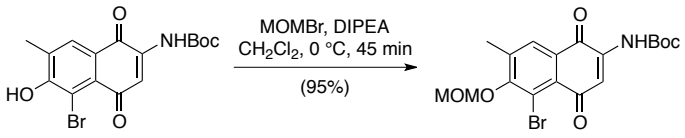
M.p.: 173 °C

^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.93 (s, 1 H), 7.64 (s, 1 H), 7.38 (s, 1 H), 7.02 (s, 1 H), 2.41 (s, 3 H), 1.53 (s, 9 H).

^{13}C NMR (75 MHz, $CDCl_3$) δ: 183.3, 179.2, 156.9, 151.2, 139.7, 130.0, 129.7, 127.8, 124.9, 115.6, 109.1, 82.6, 28.1, 17.1.

IR (Diamond-ATR, neat) ν_{max}: 3376, 2983, 1740, 1657, 1647, 1622, 1581, 1507, 1457, 1327, 1271, 1231, 1206, 1181, 1144, 1094, 1042, 1011, 967, 886, 867, 816, 680 cm^{-1}.

HRMS (ESI) calcd for $C_{16}H_{15}BrNO_5$ $[M-H]^-$: 380.0139; found: 380.0141.



2.54 **2.55**

MOM-aminonaphthoquinone 2.55:

Bromo-naphthoquinone **2.54** (1.32 g, 3.46 mmol, 1.0 equiv.) was dissolved in dry CH_2Cl_2 (100 mL) and the clear orange solution was cooled to 0 °C. DIPEA (1.21 mL, 6.92 mmol, 2.0 equiv.) was added dropwise, upon which the solution rapidly turned dark purple. Bromomethyl methyl ether (367 µL, 4.50 mmol, 1.3 equiv.) was added dropwise and the reaction mixture was allowed to stir for 45 min at 0 °C. Sat. aq. $NaHCO_3$ (50 mL) was then added and the layers separated. The aqueous layer was extracted with $CHCl_3$ (3 × 100 mL), the combined organics were dried over sodium sulfate, filtered and the solvent was removed

under reduced pressure. Purification of the residue by flash column chromatography (silica gel, gradient: $CHCl_3$:acetone = 40:1 → 30:1) afforded MOM-ether **2.55** (1.40 g, 3.29 mmol, 95%) as a yellow solid.

TLC ($CHCl_3$:acetone = 20:1), R_f = 0.63 (UV/CAM)

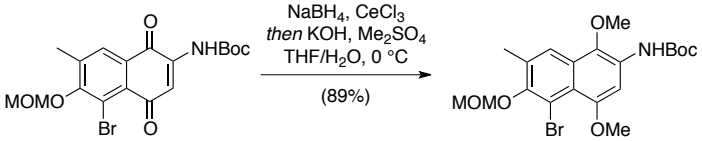
M.p.: 42 °C

^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.99 (d, *J* = 0.7 Hz, 1 H), 7.59 (*br* s, 1 H), 7.43 (s, 1 H), 5.18 (s, 2 H), 3.67 (s, 3 H), 2.49 (d, *J* = 0.7 Hz, 3 H), 1.53 (s, 9 H).

^{13}C NMR (75 MHz, $CDCl_3$) δ: 183.2, 179.6, 160.5, 151.2, 139.3, 138.4, 129.7, 129.1, 128.4, 118.0, 116.7, 100.4, 82.6, 58.0, 28.1, 17.9.

IR (Diamond-ATR, neat) ν_{max}: 3384, 2977, 2931, 1735, 1666, 1646, 1629, 1582, 1501, 1454, 1392, 1368, 1327, 1271, 1239, 1208, 1140, 1100, 1078, 1040, 1005, 971, 918, 884, 864, 804, 751, 726 cm^{-1}.

HRMS (ESI) calcd for $C_{18}H_{21}BrNO_6$ $[M+H]^+$: 426.0547; found: 426.0549.



2.55 **2.38**

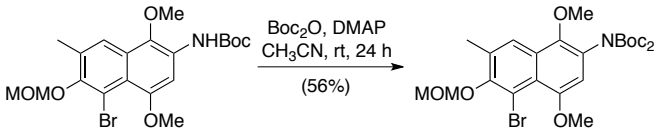
Hydroquinone dimethyl ether 2.56:

MOM-ether **2.55** (1.40 g, 3.29 mmol, 1.0 equiv.) was dissolved in a 2:1 mixture of THF (80 mL) and water (40 mL) and Cerium(III)-chloride heptahydrate (1.80 g, 4.94 mmol, 1.50 equiv.) was added. The reaction mixture was cooled to 0 °C and $NaBH_4$ (250 mg, 6.58 mmol, 2.0 equiv.) was added in two portions over 5 min. After 20 min, a solution of KOH (3.70 g, 65.8 mmol, 20 equiv.) in H_2O (15 mL) was added dropwise to the white reaction mixture which turned immediately black-brown. Me_2SO_4 (9.3 mL, 98.7 mmol, 30 equiv.) was next added dropwise and the resulting solution was stirred at 0 °C for 35 min, after which conc. NH_4OH (50 mL) and H_2O (15 mL) were added. The reaction mixture was warmed to rt, extracted with EtOAc (3 × 250 mL) and the combined organic layers were dried over sodium sulfate, filtered and concentrated under reduced pressure. Purification of the residue by flash column chromatography (silica gel, gradient: hexanes:EtOAc = 20:1 → 10:1→ 5:1) afforded the title compound **2.38** (1.33 g, 2.92 mmol, 89%) as an off-white solid.

TLC (hexanes:EtOAc = 2:1), R_f = 0.77 (UV/CAM)

M.p.: 100 °C

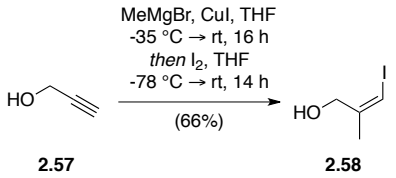
^{1}H NMR (600 MHz, $CDCl_3$) δ: 7.87 (*br* s, 1 H), 7.70 (d, *J* = 1.0 Hz, 1 H), 7.11 (*br* s, 1 H), 5.16 (s, 2 H), 3.96 (s, 3 H), 3.82 (s, 3 H), 3.68 (s, 3 H), 2.53 (d, *J* = 0.9 Hz, 3 H), 1.56 (s, 9 H).
^{13}C NMR (150 MHz, $CDCl_3$) δ: 152.7, 152.5, 152.3, 134.8, 133.0, 128.2, 127.3, 122.0, 120.0, 111.8, 100.5, 100.1, 80.9, 61.3, 57.8, 56.0, 28.4, 18.4.
IR (Diamond-ATR, neat) ν_{max}: 3308, 2977, 2928, 1718, 1626, 1614, 1573, 1512, 1448, 1362, 1315, 1264, 1243, 1221, 1148, 1112, 1043, 984, 940, 916, 874, 830, 783, 769, 761, 669 cm^{-1}.
HRMS (EI) calcd for $C_{20}H_{26}BrNO_6$ $[M]^{+\bullet}$: 455.0944; found: 455.0942.



2.38 **2.56**

Di-Boc-naphthalene 2.56:

To a solution of hydroquinone dimethyl ether **2.38** (32 mg, 0.07 mmol, 1.0 equiv.) in dry CH_3CN (3 mL) was added Boc_2O (23 mg, 0.11 mmol, 1.5 equiv.) and DMAP (11 mg, 0.09 mmol, 1.3 equiv.) and the reaction mixture was stirred at rt for 24 h. Sat. aq. $NaHCO_3$ (2 mL) was added and the reaction mixture was extracted with $CHCl_3$ (3 × 10 mL). The combined organics were dried over sodium sulfate, filtered and concentrated *in vacuo*. Purification of the residue by flash column chromatography (silica gel, gradient: hexanes:EtOAc = 15:1 → 7:1) afforded the title compound **2.56** (22 mg, 39.5 µmol, 56%) as a colourless wax.
TLC (hexanes:EtOAc = 5:1), R_f = 0.37 (UV/CAM)
^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.89 (d, *J* = 1.0 Hz, 1 H), 6.60 (s, 1 H), 5.18 (s, 2 H), 3.90 (s, 3 H), 3.83 (s, 3 H), 3.69 (s, 3 H), 2.54 (d, *J* = 0.8 Hz, 3 H), 1.41 (s, 18 H).
^{13}C NMR (100 MHz, $CDCl_3$) δ: 154.0, 151.8, 151.5, 144.1, 132.8, 128.1, 127.9, 123.9, 123.7, 111.6, 108.1, 100.1, 82.7, 61.4, 57.9, 56.2, 27.9, 18.4.
IR (Diamond-ATR, neat) ν_{max}: 3428, 2980, 2936, 2254, 1782, 1747, 1711, 1622, 1606, 1573, 1458, 1409, 1392, 1369, 1319, 1276, 1252, 1150, 1101, 1046, 984, 947, 852, 751, 733, 666 cm^{-1}.
HRMS (ESI) calcd for $C_{25}H_{34}BrNO_8Na$ $[M+Na]^+$: 578.1360; found: 578.1371.

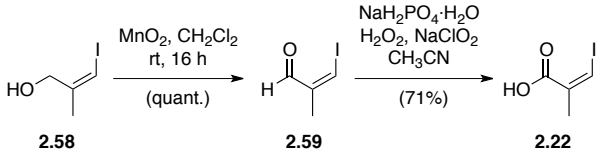


(*Z*)-3-iodo-2-methylprop-2-en-1-ol (2.58)

To a suspension of copper(I)-iodide (1.90 g, 10 mmol, 0.1 equiv.) in dry THF (160 mL) was added propargyl alcohol (**2.57**) (5.61 g, 100 mmol, 1.0 equiv.) and the resulting white suspension was cooled to -35 °C. Methylmagnesium bromide (3 M solution in Et_2O, 84 mL, 2.5 equiv.) was added by syringe pump over 2 h and the mixture was warmed to rt and stirred for 16 h. Then, the dark-grey reaction mixture was cooled to -78 °C and a solution of iodine (28 g, 110 mmol, 1.1 equiv.) in dry THF (80 mL) was added via cannula over 20 min. The reaction mixture was warmed to rt, stirred for 14 h and then quenched with sat. aq. solution of NH_4Cl (150 mL). The layers were separated and the aq. layer was extracted with Et_2O (3 × 150 mL). The combined org. extracts were dried over sodium sulfate, filtered and concentrated *in vacuo* and the resulting crude was purified by flash column chromatography (silica gel, hexanes:EtOAc = 4:1) to give allylic alcohol **2.58** (13 g, 65.6 mmol, 66%) as a brown oil.

TLC (hexanes:EtOAc = 4:1), R_f = 0.32 (UV/$KMnO_4$)

^{1}H NMR (200 MHz, $CDCl_3$) δ: 5.99–5.97 (m, 1 H), 4.25 (s, 2 H), 1.99–1.98 (m, 3 H).



(*Z*)-3-iodo-2-methylacrylic acid (2.22)

To a solution of alcohol **2.58** (2.0 g, 10.1 mmol, 1.0 equiv.) in dry CH_2Cl_2 (200 mL) was added manganese dioxide (17.6 g, 202 mmol, 20 equiv.) at rt. The resulting mixture was stirred for 16 h and filtered over a pad of Celite. Careful concentration (500 mbar/40 °C water bath) of the collected filtrate gave sensitive aldehyde **2.59** (2.0 g, 10.2 mmol, quant.), which was directly used in the next step without further purification.

Crude aldehyde **2.59** (2.0 g, 10.2 mmol, 1.0 equiv.) was dissolved in CH_3CN (12 mL) and a solution of $NaH_2PO_4·H_2O$ (424 mg, 3.07 mmol, 0.3 equiv.) in water (8 mL) was added. The mixture was cooled to 0 °C and H_2O_2 (14 mL) was added. Then, a solution of $NaClO_2$ (1.6 g, 17.7 mmol, 1.7 equiv.) was added dropwise and the reaction mixture was stirred at 0 °C for 1

h before being warmed to rt. After 2 h, the mixture was extracted with Et_2O (3 × 25 mL), the aq. layer was acidified with 2 M HCl and extracted with Et_2O (3 × 25 mL). The combined org. layers were dried over sodium sulfate, filtered and concentrated to afford a light yellow liquid. Purification of the crude product by flash column chromatography (silica gel, pentane:Et_2O = 1:1) afforded vinyl iodide **2.22** (1.55 g, 7.3 mmol, 71%) as a colourless liquid which solidified in the freezer.

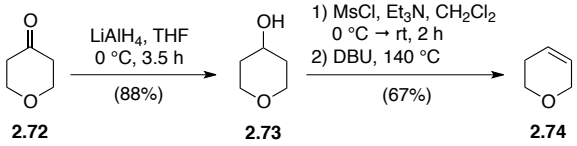
TLC (hexanes:EtOAc:AcOH = 1:1:0.01), R_f = 0.36 (UV/$KMnO_4$)

^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.12 (q, *J* = 1.5 Hz, 1 H), 2.11 (d, *J* = 1.6 Hz, 3 H).

^{13}C-NMR (100 MHz, $CDCl_3$) δ = 171.7, 137.7, 86.3, 22.4.

HRMS (EI) calcd for $C_4H_5IO_2$ $[M]^{+\bullet}$: 211.9334; found: 211.9331.

3.3.2 Divergolides C and D Project



3,6-Dihydro-2*H*-pyran (2.74):

Tetrahydro-4*H*-pyran-4-one (**2.72**) (2.5 g, 25 mmol, 1.0 equiv.) was dissolved in dry THF (13 mL) and cooled to 0 °C. In a separate flask, $LiAlH_4$ (1.9 g, 50 mmol, 2.0 equiv.) was suspended in dry THF (25 mL) and the slurry was slowly transferred via cannula to the flask containing pyranone **2.72**. The reaction mixture was stirred for 3.5 h at 0 °C and subsequently quenched by addition of water (2 mL) and NaOH (3 M, 2 mL). The suspension was filtered through Celite and the filter cake was washed with EtOAc (2 × 70 mL). The filtrate was concentrated *in vacuo* to give crude alcohol **2.73** (2.25 g, 22.0 mmol, 88%), which was used in the next step of the reaction sequence without further purification.

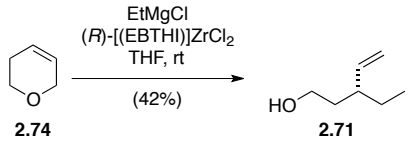
To a solution of the crude pyranol **2.73** (2.25 g, 22.0 mmol, 1.0 equiv.) in dry CH_2Cl_2 (45 mL) was added triethylamine (3.69 mL, 26.4 mmol, 1.2 equiv.) and the clear solution was cooled to 0 °C. Methanesulfonyl chloride (1.86 mL, 24.0 mmol, 1.09 equiv.) was then added dropwise and the resulting cloudy mixture was stirred at 0 °C for 1 h, warmed to rt and stirred for another 2 h, before water (40 mL) was added. The organic layer was separated, dried over sodium sulfate, filtered and concentrated under reduced pressure. To the yellow crude mesylate was transferred to a flask fitted for distillation and then DBU (3.75 mL) was added. The flask reaction mixture was slowly heated to 140 °C and the material boiling at 68-70 °C was collected to provide pyran **2.74** (1.24 g, 14.7 mmol, 67%) as a volatile, colorless liquid.

TLC (hexanes:EtOAc = 2:1), R_f = 0.67 ($KMnO_4$)

^{1}H NMR (400 MHz, CD_2Cl_2) δ: 5.85–5.80 (m, 1 H), 5.72–5.68 (m, 1 H), 4.08–4.05 (m, 2 H), 3.73 (t, *J* = 5.5 Hz, 2 H), 2.12–2.06 (m, 2 H).

^{13}C NMR (100 MHz, CD_2Cl_2) δ: 127.1, 124.7, 65.7, 64.6, 25.8.

HRMS (EI) calcd for C_5H_8O $[M]^{+\bullet}$: 84.0575; found: 84.0568.



2.74 **2.71**

(*R*)-3-Vinyl-pentan-1-ol (2.71):

A Schlenk flask was charged with the catalyst (*R*)-[(EBTHI)]$ZrCl_2$ (37.0 mg, 86.7 µmol, 0.1 equiv.), dry THF (1 mL) and EtMgCl (2 M solution in THF, 2.6 mL, 5.2 mmol, 5.8 equiv.) and the resulting yellow solution was stirred under argon at rt for 45 min. In a separate flask, EtMgCl (2 M solution in THF, 0.45 mL, 0.9 mmol, 1.0 equiv.) was added to a solution of 3,4-dihydropyran (**2.74**) (76.0 mg, 0.90 mmol, 1.0 equiv.) in dry THF (0.4 mL). The latter solution was next transferred to the solution containing the Zr-catalyst via cannula and the solution was stirred at rt. After 16 h, the reaction mixture was cooled to 0 °C and quenched by dropwise addition of aq. HCl (0.1 M, 2 mL). Water (10 mL) and CH_2Cl_2 (15 mL) were added and the mixture was filtered over a short plug of Celite. The organic layer of the filtrate was separated, dried over sodium sulfate, filtered and concentrated *in vacuo*. Purification of the yellow crude product by flash column chromatography (silica gel, hexanes:EtOAc = 2:1) afforded the title compound **2.71** (43.1 mg, 0.38 mmol, 42%) as a colorless liquid.

TLC (hexanes:EtOAc = 2:1), R_f = 0.42 ($KMnO_4$)

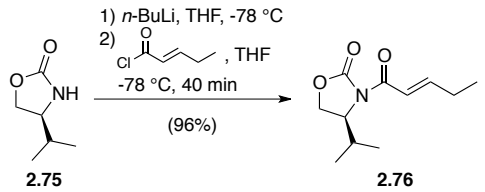
^{1}H NMR (600 MHz, $CDCl_3$) δ: 5.59–5.53 (m, 1 H), 5.03–5.02 (m, 1 H), 5.01–5.00 (m, 1 H), 3.70–3.61 (m, 2 H), 2.07–2.01 (m, 1 H), 1.71–1.65 (m, 1 H), 1.53–1.47 (m, 1 H), 1.45–1.39 (m, 1 H), 1.32–1.25 (m, 1 H), 0.86 (t, *J* = 7.4 Hz, 3 H).

^{13}C-NMR (75 MHz, $CDCl_3$) δ = 142.7, 114.9, 61.4, 42.9, 37.5, 27.9, 11.5.

IR (Diamond-ATR, neat) ν_{max}: 3324, 2961, 2923, 2875, 1641, 1455, 1420, 1379, 1292, 1237, 1201, 1057, 1023, 995, 910, 874, 734, 675 cm^{-1}.

HRMS (EI) calcd for $C_7H_{14}O$ $[M]^{+\bullet}$: 114.1045; found: 114.1043.

$[\alpha]^{25}_D$ = –12.4° (*c* = 0.5, $CHCl_3$)



Enone 2.76:

A solution of (*S*)-4-isopropyloxazolidin-2-one (**2.75**) (3.31 g, 25.6 mmol, 1.0 equiv.) in dry THF (80 mL) was cooled to -78 °C and *n*-BuLi (2.5 M in hexanes, 10.4 mL, 25.9 mmol, 1.03 equiv.) was added dropwise over 10 min. Then, (*E*)-pent-2-enoyl chloride[48] (3.34 g, 28.2 mmol, 1.1 equiv.) was added in one portion and the resulting yellow solution was continued to be stirred at -78 °C. After 40 min, the reaction mixture was warmed to rt over 20 min and subsequently quenched by addition of sat. aq. solution of NH_4Cl (25 mL). The volatiles (THF and hexanes) were removed under reduced pressure and the residue was extracted with CH_2Cl_2 (3 × 50 mL). The combined organics were washed with NaOH (1 M, 75 mL), sat. aq. NaCl (100 mL), dried over sodium sulfate, filtered and concentrated *in vacuo*. Purification of the crude yellow oil by flash column chromatography (silica gel, hexanes:EtOAc = 10:1) afforded the title compound **2.76** (5.15 g, 24.4 mmol, 95%) as a white solid.

TLC (hexanes:EtOAc = 10:1), R_f = 0.19 (UV/$KMnO_4$)

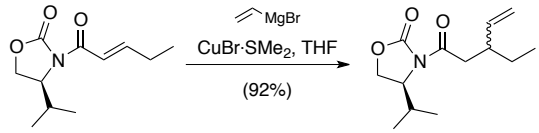
^{1}H NMR (600 MHz, $CDCl_3$) δ: 7.26 (dt, *J* = 15.4, 1.4 Hz, 1 H), 7.18 (dt, *J* = 15.4, 6.2 Hz, 1 H), 4.49 (ddd, *J* = 8.3, 3.9, 3.1 Hz, 1 H), 4.29–4.26 (m, 1 H), 4.21 (dd, *J* = 9.1, 3.1 Hz, 1 H), 2.45–2.37 (m, 1 H), 2.30 (qdd, *J* = 7.5, 6.2, 1.4 Hz, 2 H), 1.10 (t, *J* = 7.4 Hz, 3 H), 0.92 (d, *J* = 7.1 Hz, 3 H), 0.88 (d, *J* = 6.9 Hz, 3 H).

^{13}C NMR (150 MHz, $CDCl_3$) δ: 165.2, 154.1, 152.8, 119.6, 63.3, 58.5, 28.5, 25.8, 18.0, 14.7, 12.2.

IR (Diamond-ATR, neat) ν_{max}: 2966, 2935, 2877, 1770, 1681, 1635, 1487, 1466, 1386, 1363, 1349, 1298, 1260, 1199, 1146, 1121, 1096, 1062, 1042, 1016, 974, 858, 773, 752, 713, 665 cm^{-1}.

HRMS (EI) calcd for $C_{11}H_{17}NO_3$ $[M]^{+\bullet}$: 211.1208; found: 211.1189.

$[\alpha]^{25}_D$ = +100.4° (*c* = 0.5, $CHCl_3$)



2.76 **2.77**

Imide 2.77:

To a suspension of $CuBr{\cdot}SMe_2$ (2.92 g, 14.2 mmol, 1.5 equiv.) and 4 Å mol sieves in dry THF (100 mL) in a Schlenk flask was added dimethyl sulfide (38 mL) at rt. The solution was cooled to -48 °C and vinylmagnesium bromide (31.6 mL, 28.4 mmol, 3.0 equiv.) was added. After 10 min stirring at -23 °C, a solution of enone **2.76** (2.0 g, 9.48 mmol, 1.0 equiv.) in dry THF (40 mL) was added and the whole was stirred at this temperature for 1 h. Then, the reaction was quenched with sat. aq. solution of NH_4Cl (100 mL) and warmed to rt. The reaction mixture was filtered through a pad of Celite, and the filtrate was extracted with EtOAc (3 × 100 mL). The combined organic layers were washed with sat. aq. NaCl (250 mL), dried over sodium sulfate, filtered and concentrated *in vacuo*. The yellow residue was purified by flash column chromatography (silica gel, hexanes:EtOAc = 15:1) to provide product **2.77** (2.09 g, 8.76 mmol, 92%) as a 2:1 mixture of diastereomers.

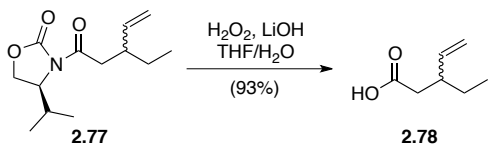
TLC (hexanes:EtOAc = 5:1), $R_f = 0.39$ ($KMnO_4$)

Major diastereomer:

^{1}H NMR (300 MHz, $CDCl_3$) δ: 5.67 (ddd, J = 17.2, 10.3, 8.5 Hz, 1 H), 5.06–4.98 (m, 2 H), 4.45–4.40 (m, 1 H), 4.27–4.16 (m, 2 H), 3.14 (dd, J = 15.6, 8.5 Hz, 1 H), 2.89 (dd, J = 16.2, 7.4 Hz, 1 H), 2.57–2.49 (m, 1 H), 2.40–2.28 (m, 1 H), 1.53–1.31 (m, 3 H), 0.90 (d, J = 7.2 Hz, 3 H), 0.85 (d, J = 6.9 Hz, 3 H).

^{13}C NMR (75 MHz, $CDCl_3$) δ: 172.2, 154.0, 140.9, 115.2, 63.2, 58.5, 41.9, 40.0, 28.3, 27.5, 18.0, 14.6, 11.5.

HRMS (EI) calcd for $C_{13}H_{21}NO_3$ $[M]^{+\bullet}$: 239.1521; found: 239.1507.



2.77 **2.78**

Acid 2.78:

A solution of imide **2.77** (280 mg, 1.17 mmol, 1.0 equiv.) in THF (16 mL) and water (5 mL) was cooled to 0 °C and H_2O_2 (6.5 mL) added. The mixture was stirred at this temperature for 2 min, $LiOH{\cdot}H_2O$ (97 mg, 2.31 mmol, 2.0 equiv.) was added and the reaction mixture was

warmed to rt. After 24 h, the solution was cooled back to 0 °C and aq. sodium sulfite solution (1.5 M, 3.5 mL) was added. Stirring was continued for 1 h, before THF was removed in under reduced pressure. The residue was extracted with CH_2Cl_2 (2 × 10 mL), the aqueous layer was acidified to pH = 1 with aq. HCl (1 M) and then extracted extensively extracted with CH_2Cl_2 and EtOAc until TLC indicated that no more product was left in the aqueous phase. The combined organic extracts were dried over sodium sulfate, filtered and concentrated *in vacuo*. The residue was purified by flash column chromatography (silica gel, hexanes:Et_2O = 2:1) to give the title compound **2.78** (140 mg, 1.09 mmol, 93%) as a colorless liquid.

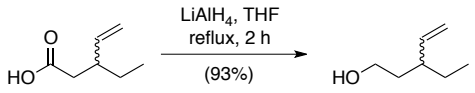
TLC (hexanes:EtOAc = 1:1), R_f = 0.52 ($KMnO_4$)

^{1}H NMR (400 MHz, CD_2Cl_2) δ: 5.65 (ddd, *J* = 17.2, 10.3, 7.8 Hz, 1 H), 5.08–5.02 (m, 2 H), 2.44–2.40 (m, 2 H), 2.36–2.32 (m, 1 H), 1.54–1.43 (m, 1 H), 1.39–1.32 (m, 1 H), 0.89 (t, *J* = 7.4 Hz, 3 H).

^{13}C NMR (100 MHz, CD_2Cl_2) δ: 178.2, 141.1, 115.4, 42.2, 39.6, 27.6, 11.6.

IR (Diamond-ATR, neat) ν_{max}: 2963, 2926, 2877, 1704, 1642, 1420, 1410, 1285, 1256, 1228, 1199, 1139, 1110, 1072, 1035, 993, 915, 771, 670 cm^{-1}.

HRMS (EI) calcd for $C_7H_{12}O_2$ $[M]^{+\bullet}$: 128.0837; found: 128.0826.



2.78 **2.79**

Alcohol 2.79:

To a solution of acid **2.78** (736 mg, 5.74 mmol, 1.0 equiv.) in dry THF (20 mL) was added $LiAlH_4$ (436 mg, 11.5 mmol, 2.0 equiv.) at 0 °C. The reaction mixture was warmed to rt, stirred for 1.5 h and then refluxed for another 2 h. Then, the mixture was cooled to rt, quenched by addition of aq. NaOH (1 M, 10 mL) and extracted with Et_2O and CH_2Cl_2 until no more product was detectable in the aqueous layer by TLC. The combined organic extracts were dried over sodium sulfate, filtered and concentrated under reduced pressure to give the title compound **2.79** (606 mg, 5.31 mmol, 93%) as a colorless, volatile liquid.

TLC (hexanes:EtOAc = 2:1), R_f = 0.41 ($KMnO_4$)

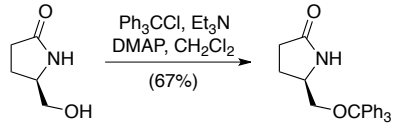
^{1}H NMR (300 MHz, $CDCl_3$) δ: 5.63–5.50 (m, 1 H), 5.05–5.03 (m, 1 H), 5.00–4.98 (m, 1 H), 3.70–3.60 (m, 2 H), 2.10–1.98 (m, 1 H), 1.73–1.63 (m, 1 H), 1.55–1.36 (m, 3 H), 1.33–1.21 (m, 1 H), 0.86 (t, *J* = 7.4 Hz, 3 H).

^{13}C NMR (75 MHz, $CDCl_3$) δ: 142.7, 114.9, 61.3, 42.9, 37.5, 27.9, 11.5.

IR (Diamond-ATR, neat) ν_{max}: 3324, 2961, 2923, 2875, 1641, 1455, 1420, 1379, 1292, 1237, 1201, 1057, 1023, 995, 910, 874, 734, 675 cm^{-1}.

HRMS (EI) calcd for $C_7H_{14}O$ $[M]^{+\bullet}$: 114.1045; found: 114.1043.

$[\alpha]^{25}_D$ = –5.4° (*c* = 0.5, $CHCl_3$)



2.80 **2.81**

(*R*)-5-((trityloxy)methyl)pyrrolidin-2-one (2.81)

To a solution of (*R*)-5-(hydroxymethyl)pyrrolidin-2-one (**2.80**) (8.04 g, 69.9 mmol, 1.0 equiv.) in dry CH_2Cl_2 (600 mL) was added triethylamine (14.6 mL, 104.9 mmol, 1.5 equiv.), DMAP (1.1 g, 9.1 mmol, 0.13 equiv.) and trityl chloride (29.2 g, 104.9 mmol, 1.5 equiv.) and the solution was stirred at rt for 12 h. The reaction mixture was washed with water (3 × 250 mL) and sat. aq. NaCl (3 × 250 mL) and the combined organic layers were dried over sodium sulfate, filtered and concentrated under reduced pressure. Purification of the residue by flash column chromatography (silica gel, gradient: CH_2Cl_2:MeOH = 50:1 → 40:1 → 10:1 → 5:1) afforded the title compound **2.81** (16.7 g, 46.6 mmol, 67%) as a white solid.

TLC ($CHCl_3$:acetone = 10:1), R_f = 0.3 (UV/CAM)

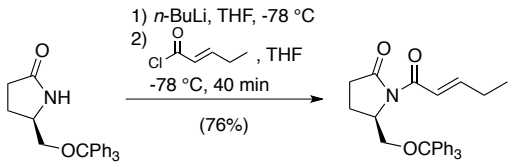
^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.44–7.41 (m, 6 H), 7.35–7.24 (m, 9 H), 6.17 (*br* s, 1 H), 3.91–3.83 (m, 1 H), 3.22 (dd, *J* = 9.3, 4.1 Hz, 1 H), 3.03 (dd, *J* = 9.3, 7.9 Hz, 1 H), 2.35–2.30 (m, 2 H), 2.21–2.09 (m, 1 H), 1.74–1.62 (m, 1 H).

^{13}C-NMR (75 MHz, $CDCl_3$) δ = 178.0, 143.6, 128.5, 127.9, 127.2, 86.8, 67.1, 54.1, 29.6, 23.3.

IR (Diamond-ATR, neat) ν_{max}: 1687, 1489, 1447, 1323, 1284, 1211, 1157, 1082, 1029, 1003, 900, 775, 760, 742, 704, 692 cm^{-1}.

HRMS (ESI) calcd for $C_{24}H_{24}NO_2$ $[M+H]^+$: 358.1802; found: 358.1802.

$[\alpha]^{25}_D$ = –14.0° (*c* = 0.2, $CHCl_3$)



2.81 **2.82**

Enone 2.82:

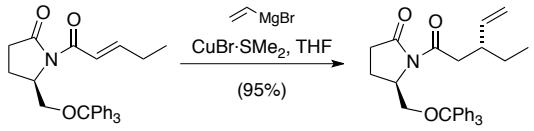
A Schlenk flask was charged with pyrrolidinone **2.81** (2.19 g, 6.13 mmol, 1.0 equiv.) and dry THF (25 mL). The solution was cooled to -78 °C and *n*-BuLi (2.5 M in hexanes, 2.48 mL, 6.19 mmol, 1.01 equiv.) was added over 5 minutes. To the resulting slightly pink solution was next added (*E*)-pent-2-enoyl chloride[48] (800 mg, 6.75 mmol, 1.1 equiv.) in one portion and stirring was continued at -78 °C for 40 minutes. The reaction mixture was then warmed to rt and quenched by addition of sat. aq. solution of NH_4Cl (6 mL). The volatiles (THF and hexanes) were removed under reduced pressure and the residue was extracted with CH_2Cl_2 (3 × 10 mL). The combined organics were washed with NaOH (1 M, 10 mL), sat. aq. NaCl (10 mL), dried over sodium sulfate, filtered and concentrated *in vacuo*. Purification of the residue by flash column chromatography (silica gel, hexanes:EtOAc = 10:1) afforded the title compound **2.82** (2.05 g, 4.66 mmol, 76%) as a white fluffy solid.

TLC (hexanes:EtOAc = 5:1), R_f = 0.47 (UV/CAM)

^{1}H NMR (600 MHz, $CDCl_3$) δ: 7.37–7.35 (m, 6 H), 7.31 (dt, *J* = 15.4, 1.7 Hz, 1 H), 7.29–7.26 (m, 6 H), 7.23–7.20 (m, 3 H), 7.13 (dt, *J* = 15.4, 6.5 Hz, 1 H), 4.56–4.51 (m, 1 H), 3.56 (dd, *J* = 9.8, 4.0 Hz, 1 H), 3.15 (dd, *J* = 9.8, 2.7 Hz, 1 H), 2.96 (ddd, *J* = 17.9, 11.1, 9.9 Hz, 1 H), 2.50 (ddd, *J* = 17.8, 9.9, 1.9 Hz, 1 H), 2.35–2.30 (m, 2 H), 2.12–2.05 (m, 1 H), 1.99–1.95 (m, 1 H), 1.13 (t, *J* = 7.4 Hz, 3 H).

^{13}C NMR (150 MHz, $CDCl_3$) δ: 176.4, 166.0, 151.9, 143.7, 128.5, 127.9, 127.1, 121.7, 87.0, 64.1, 56.8, 33.4, 25.8, 21.2, 12.5.

HRMS (ESI) calcd for $C_{29}H_{29}NO_3Na$ $[M+Na]^+$: 462.2040; found: 462.2041.



2.82 **2.83**

Imide 2.83:

To a suspension of $CuBr{\cdot}SMe_2$ (105 mg, 0.51 mmol, 1.5 equiv.) and 4 Å mol sieves in dry THF (5 mL) in a Schlenk flask was added dimethyl sulfide (1.5 mL) at rt. The resulting light-

brown solution was cooled to -48 °C and vinylmagnesium bromide (3.06 mL, 1.02 mmol, 3.0 equiv.) was added in one portion. The resulting black solution was warmed to -23 °C and continued to be stirred for further 10 min, at which point a solution of enone **2.82** (150 mg, 0.34 mmol, 1.0 equiv.) in dry THF (1.5 mL) was added. After 2.5 h at -23 °C, the reaction was quenched with sat. aq. solution of NH_4Cl (5 mL) and warmed to rt. The mixture was extracted with EtOAc (3 × 25 mL) and the organic layers were combined, dried over sodium sulfate, filtered and concentrated *in vacuo*. The residue was purified by flash column chromatography (silica gel, gradient: hexanes:EtOAc = 8:1) to provide the target compound **2.83** (152 mg, 0.33 mmol, 96%) as a single diastereomer (white solid).

TLC (hexanes:EtOAc = 5:1), R_f = 0.52 (UV/CAM)

^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.41–7.38 (m, 6 H), 7.33–7.29 (m, 6 H), 7.27–7.22 (m, 3 H), 5.71 (dd, *J* = 9.5, 7.7 Hz, 1 H), 5.05 (ddd, 16.8, 1.6, 0.8 Hz, 1 H), 5.01 (ddd, *J* = 10.3, 1.8, 0.6 Hz, 1 H), 4.50–4.46 (m, 1 H), 3.57 (dd, *J* = 9.7, 4.0 Hz, 1 H), 3.19 (dd, *J* = 9.7, 2.6 Hz, 1 H), 3.10–2.90 (m, 3 H), 2.59–2.52 (m, 1 H), 2.47 (ddd, *J* = 17.8, 9.8, 1.7 Hz, 1 H), 2.10–2.00 (m, 1 H), 1.95–1.89 (m, 1 H), 1.56–1.48 (m, 1 H), 1.42–1.30 (m, 1 H), 0.91 (t, *J* = 7.4 Hz, 3 H).

^{13}C NMR (100 MHz, $CDCl_3$) δ: 176.1, 172.6, 143.6, 141.2, 128.4, 127.8, 127.0, 114.8, 86.9, 63.8, 56.6, 41.8, 41.1, 33.1, 27.3, 21.2, 11.5.

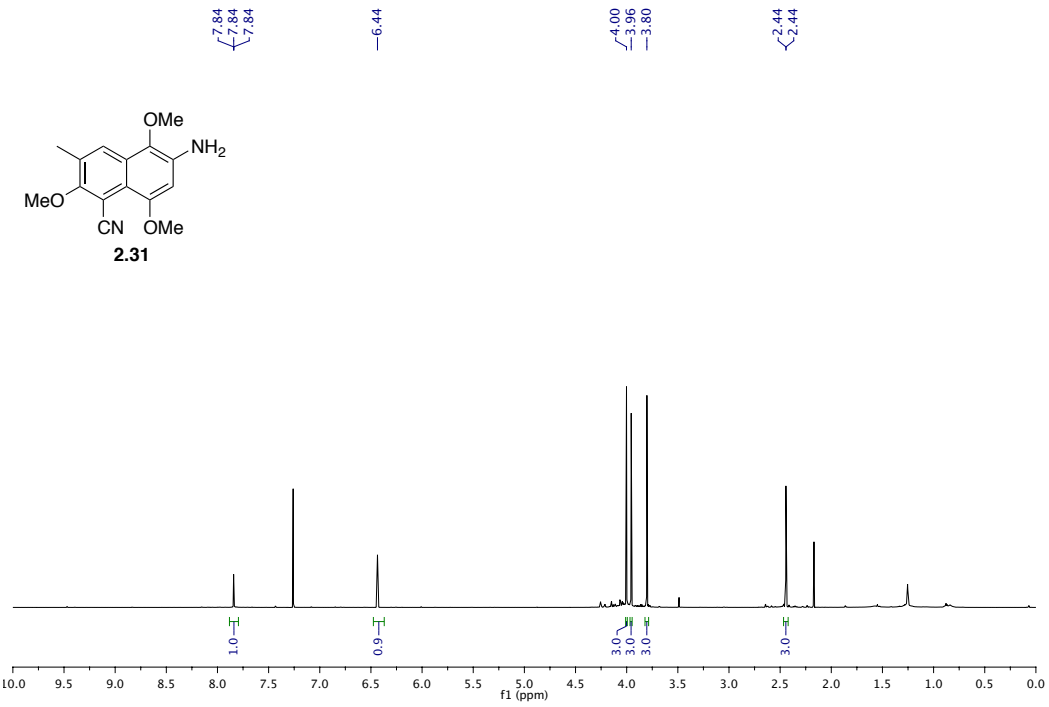
IR (Diamond-ATR, neat) ν_{max}: 3018, 2963, 1733, 1711, 1693, 1490, 1449, 1363, 1217, 1199, 1087, 1029, 1002, 916, 901, 744, 705, 666 cm^{-1}.

HRMS (ESI) calcd for $C_{31}H_{33}NO_3Na$ $[M+Na]^+$: 490.2353; found: 490.2353.

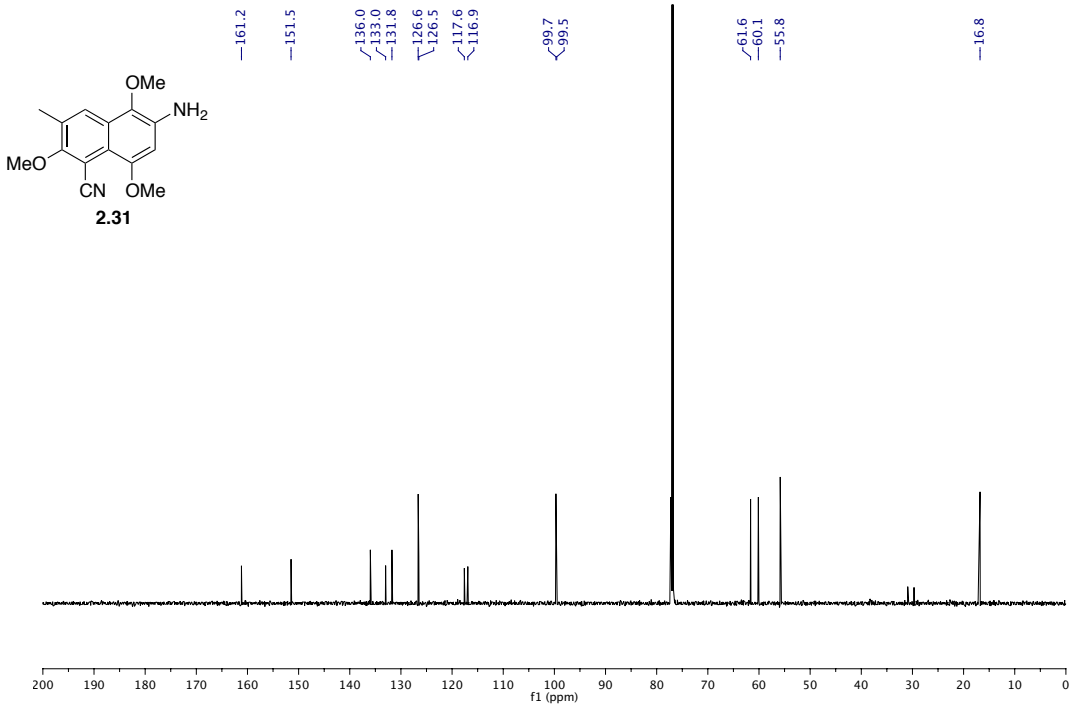
$[\alpha]^{25}_D$ = +70.4° (*c* = 0.25, $CHCl_3$)

3.4 NMR Spectra

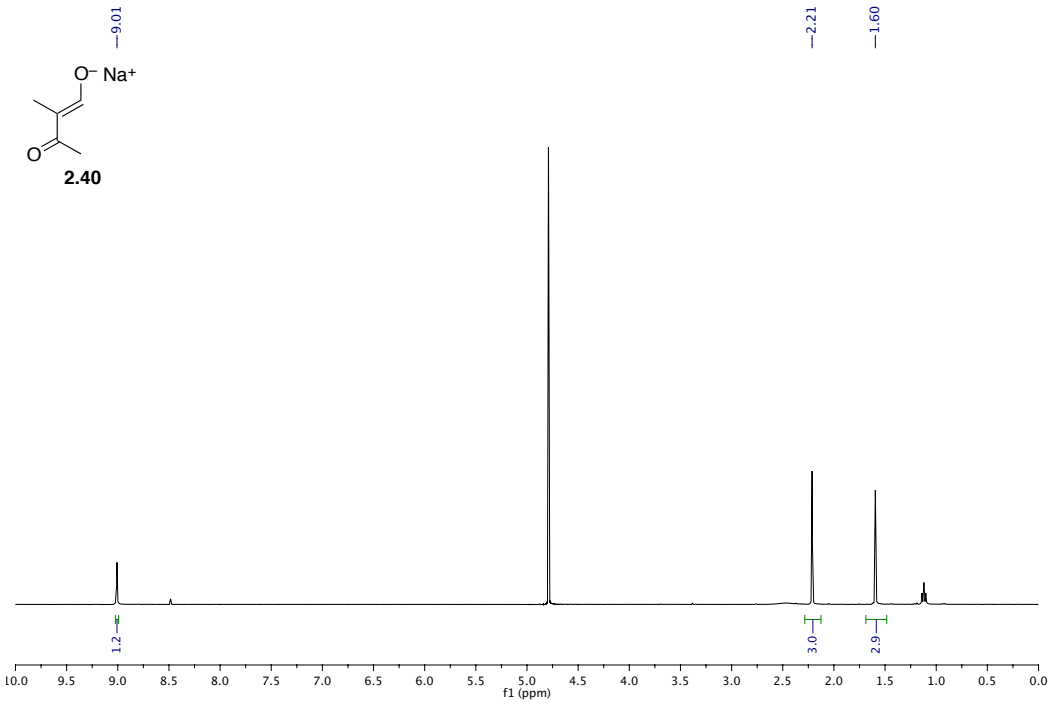
3.4.1 Naphthomycin K Project



2.31



2.31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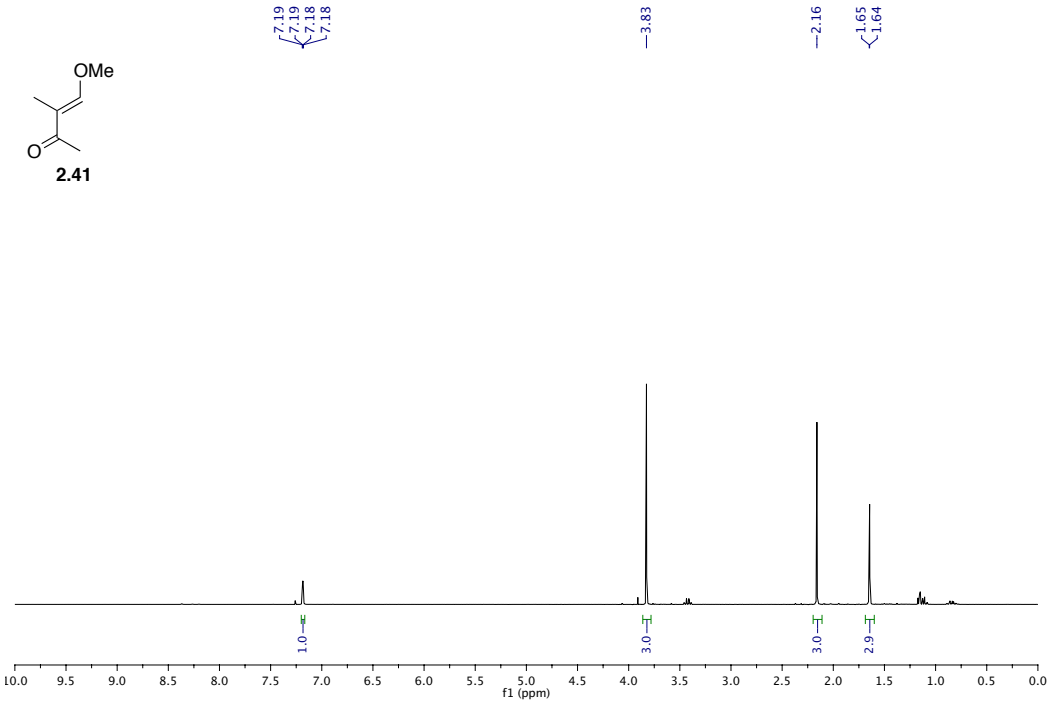
2.21
1.60
O⁻ Na⁺
O
2.40
1.2
3.0
2.9
10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)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7.18
7.18
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O
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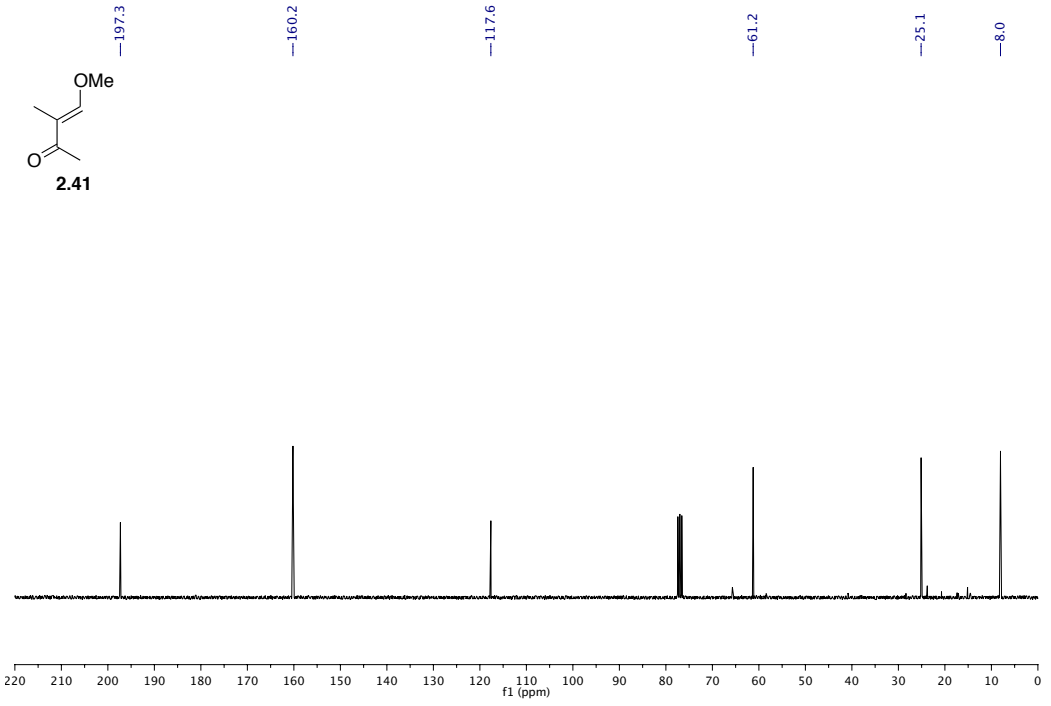
3.0
3.0
2.9
f1 (ppm)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61.2
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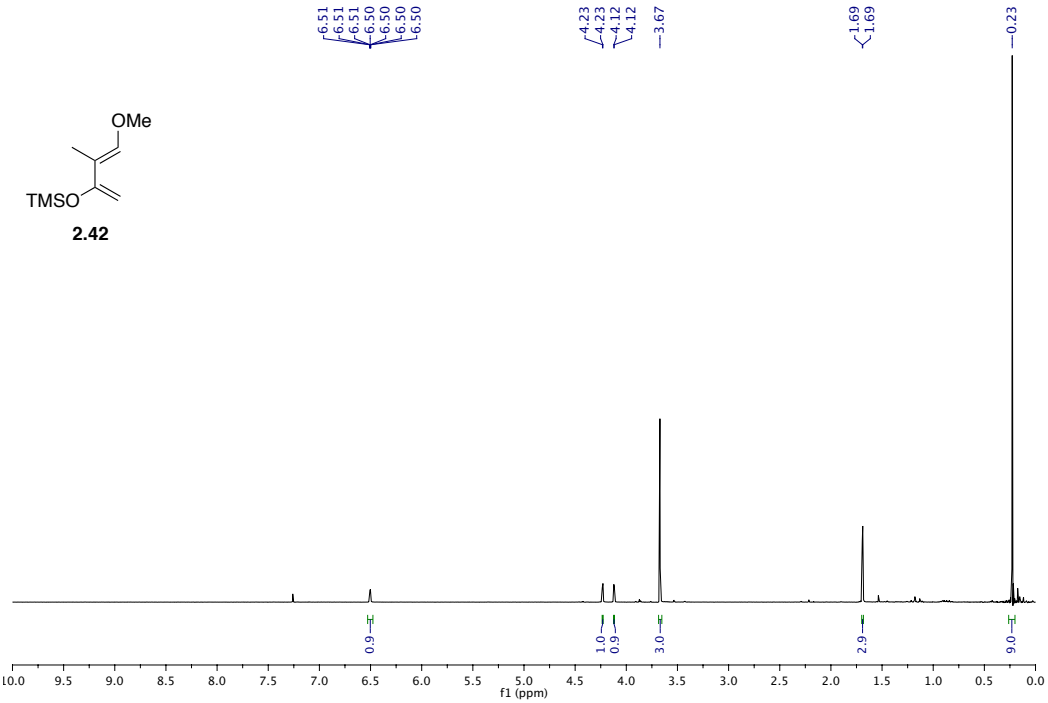
8.0
OMe
O
2.41
f1 (ppm)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6.51
6.51
6.50
6.50
6.50
6.50
4.23
4.23
4.12
4.12
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1.69
1.69
0.23
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TMSO
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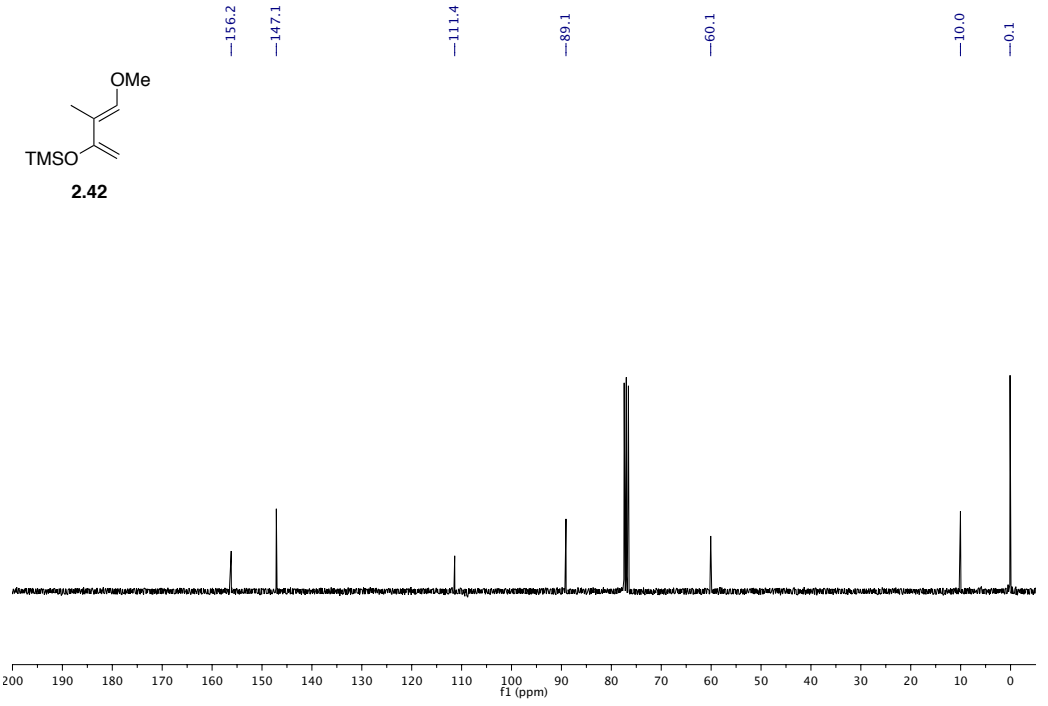
2.9
9.0
f1 (ppm)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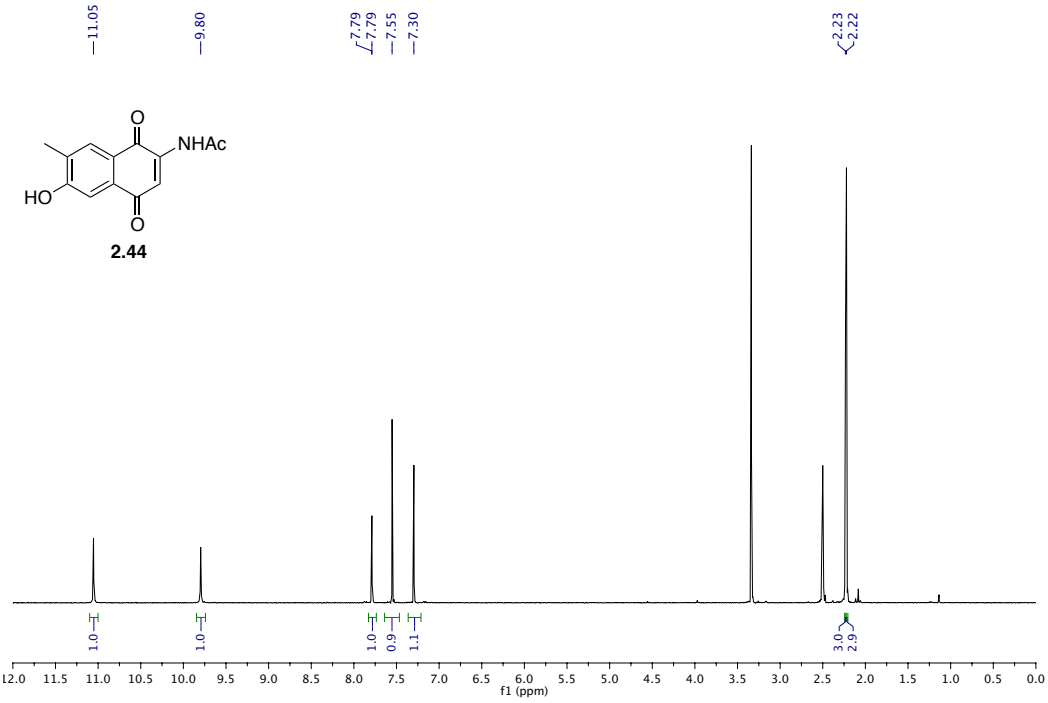
0.1
OMe
TMSO
2.42
f1 (ppm)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7.79
7.55
7.30
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2.22
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HO
O
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1.0
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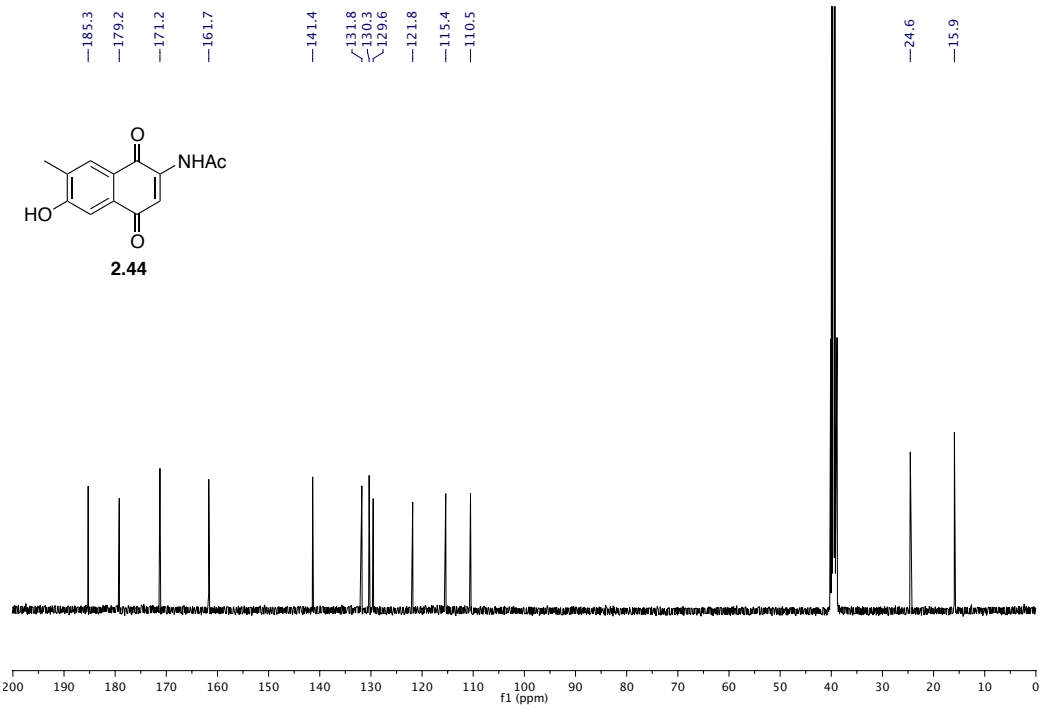
1.1
3.0
2.9
f1 (ppm)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171.2
161.7
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O
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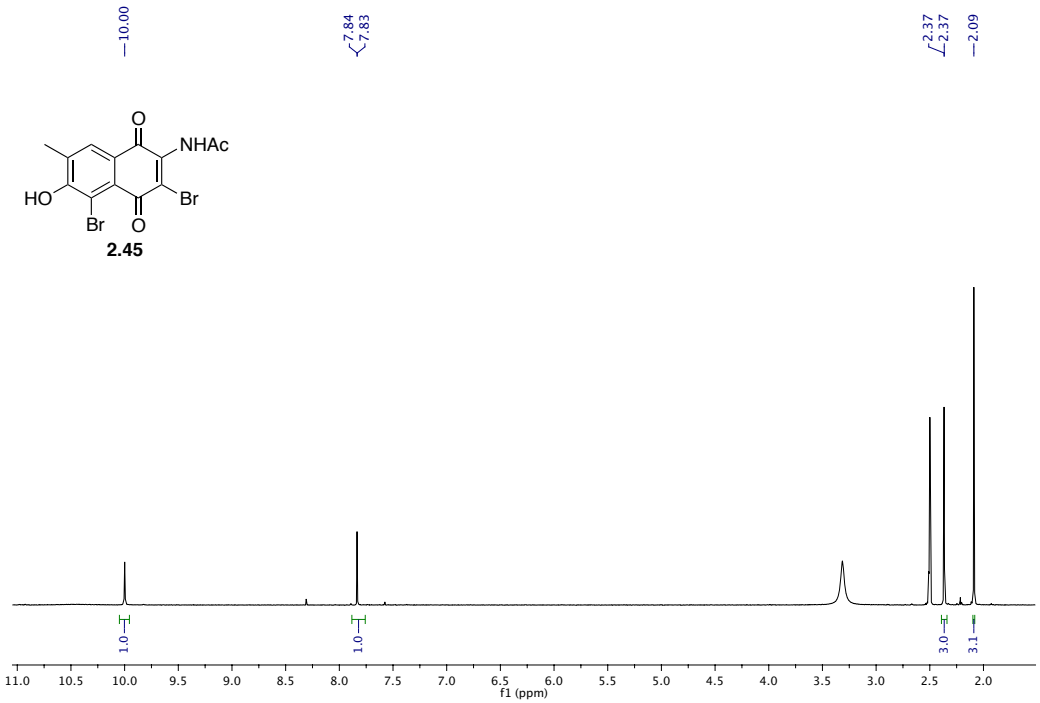
f1 (ppm)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Br
O
2.45
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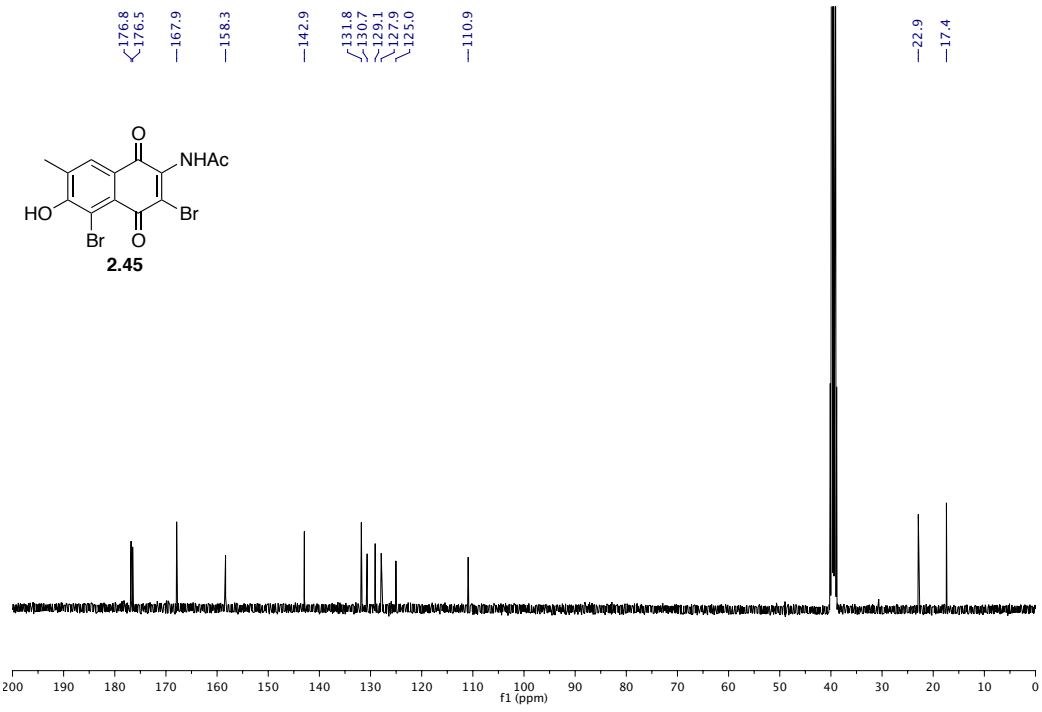
3.0
3.1
11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0
f1 (ppm)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176.5
167.9
158.3
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129.1
127.9
125.0
110.9
22.9
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HO
Br
Br
O
2.45
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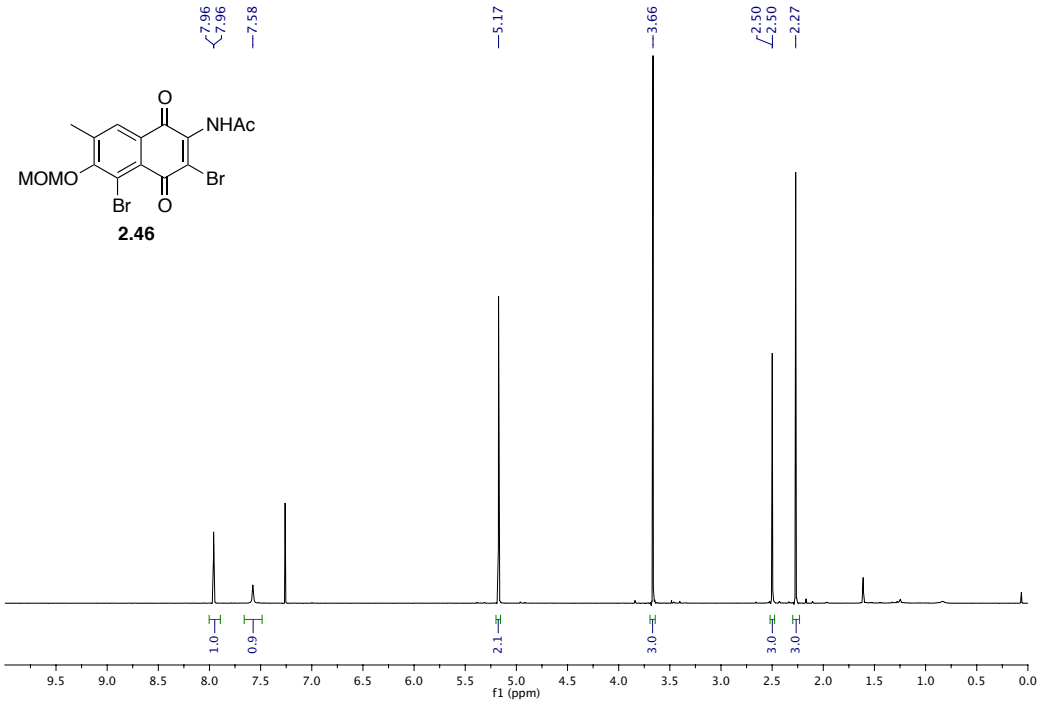
f1 (ppm)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Br
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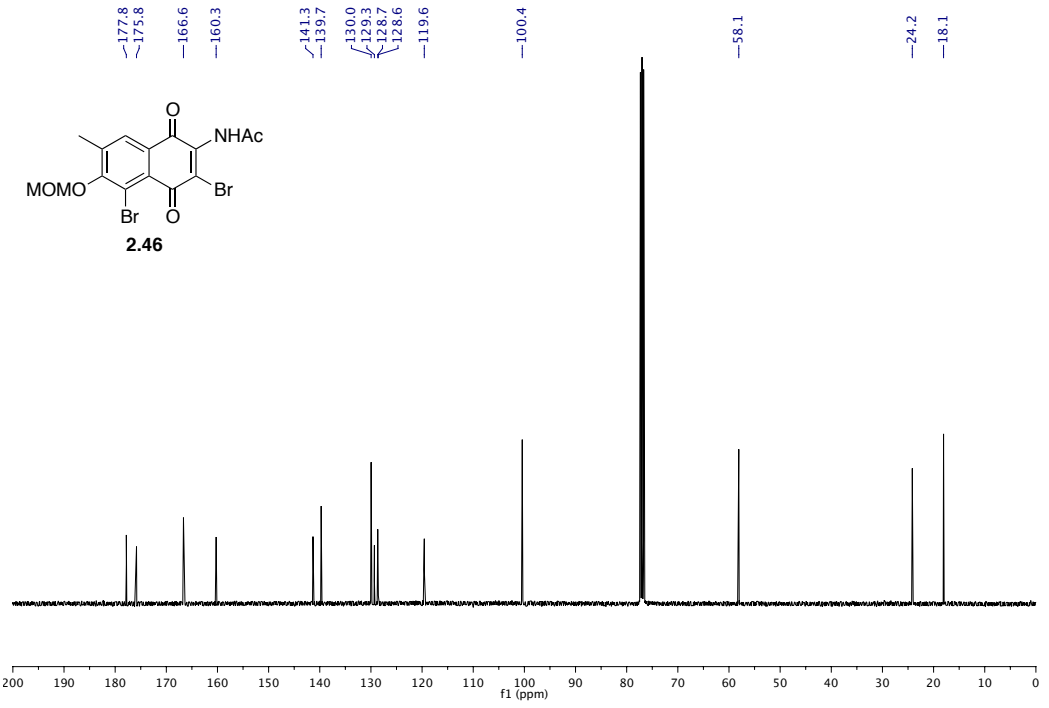
2.1
3.0
3.0
3.0
9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)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128.6
119.6
100.4
58.1
24.2
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Br
Br
O
2.46
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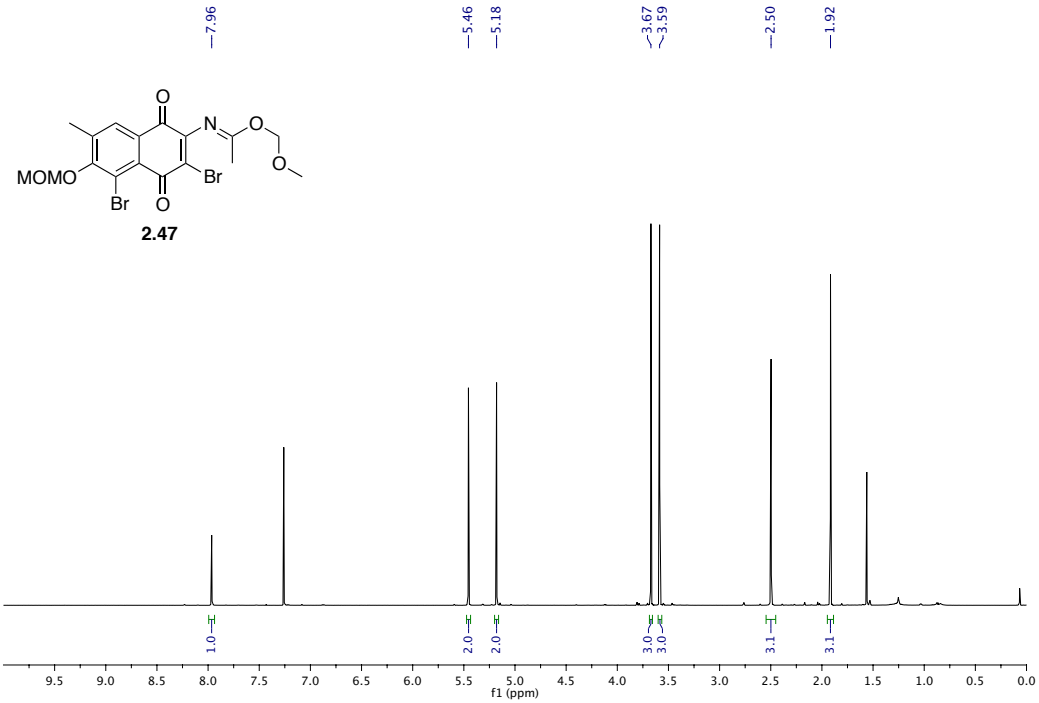
f1 (ppm)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Br
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N
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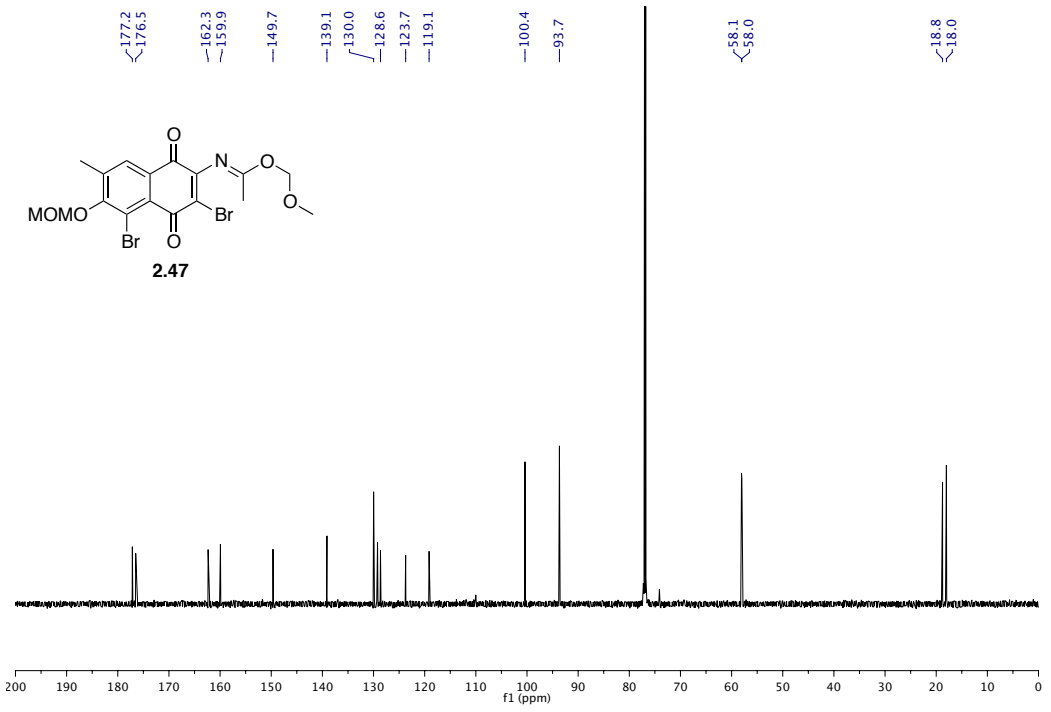
3.0
3.1
3.1
f1 (ppm)

2.47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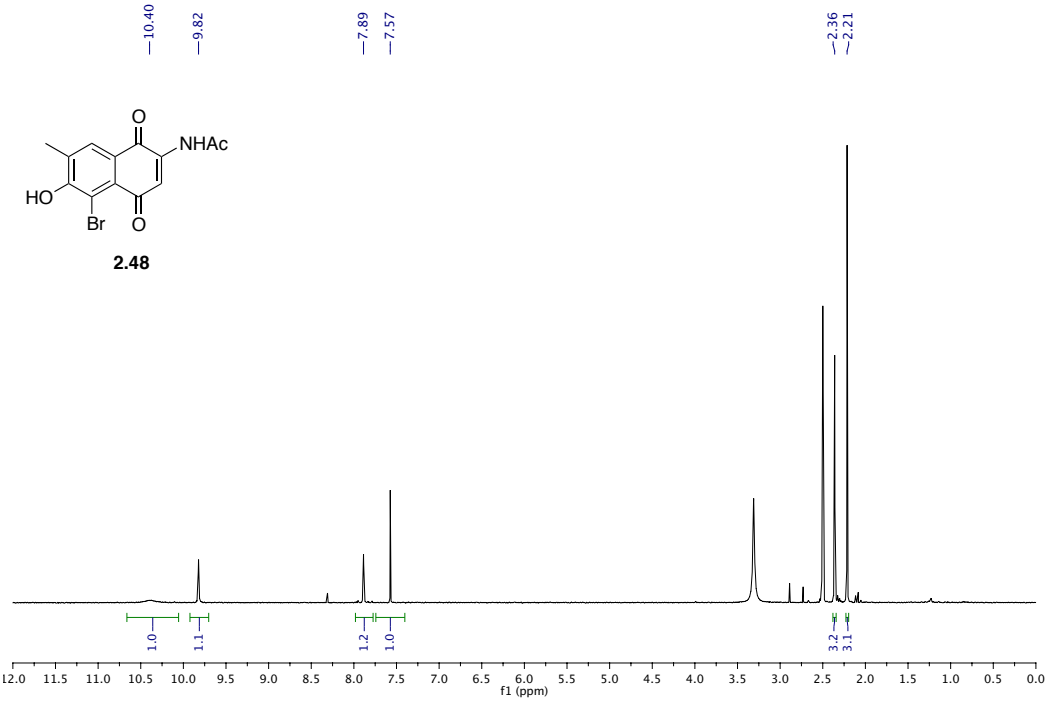
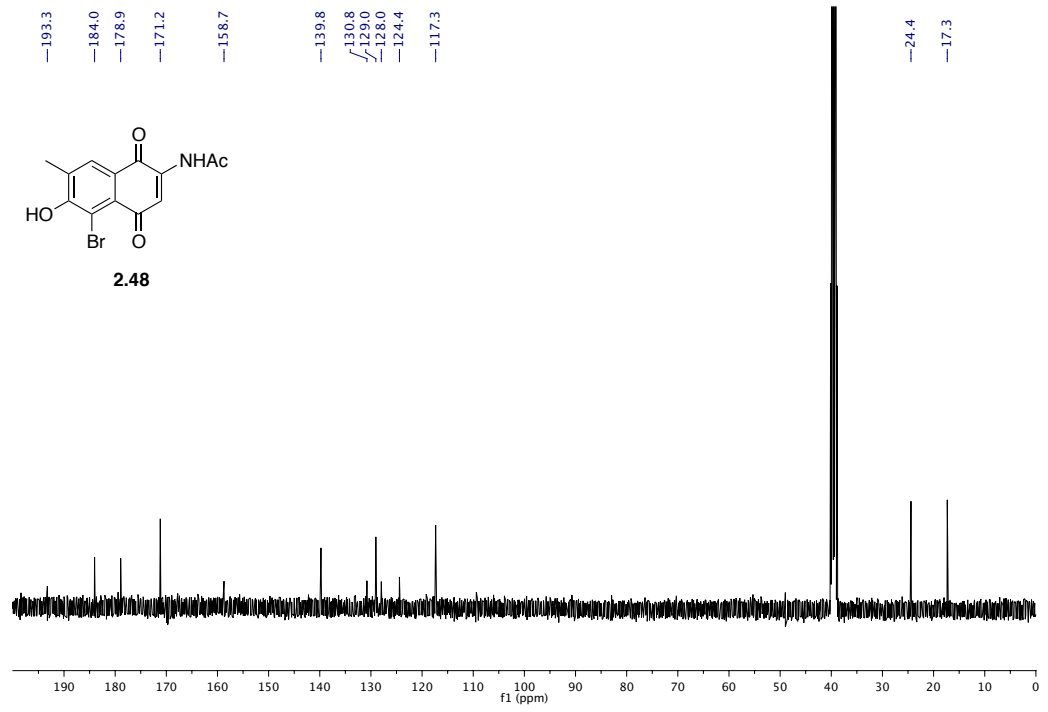
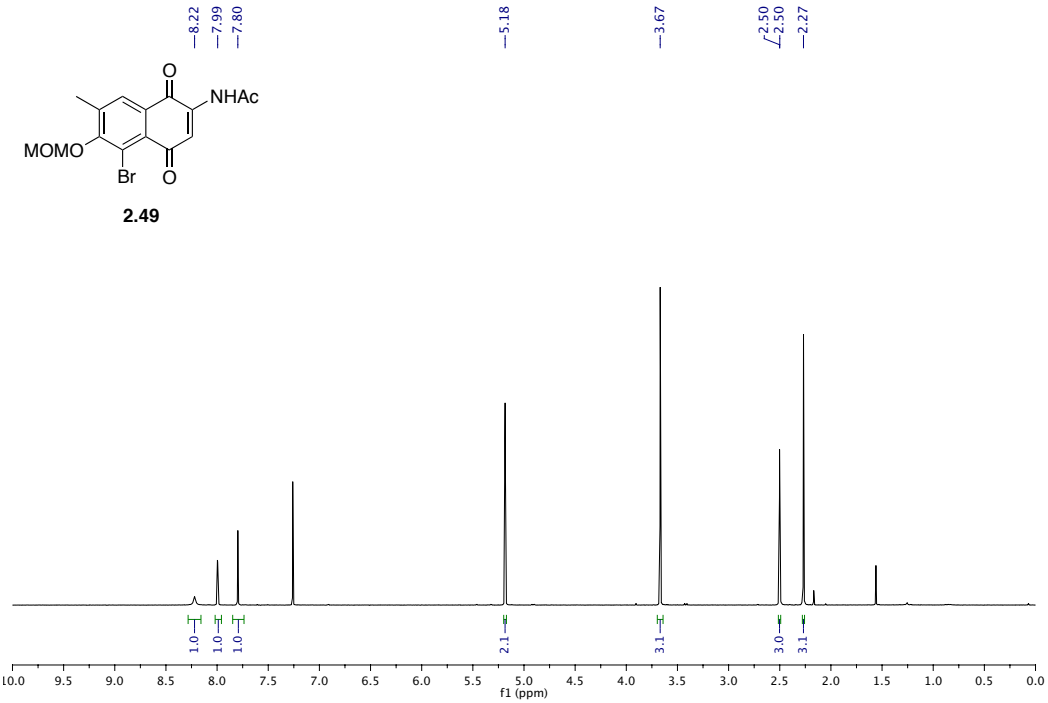
58.0
18.8
18.0
MOMO
Br
Br
O
N
O
O
O
f1 (ppm)

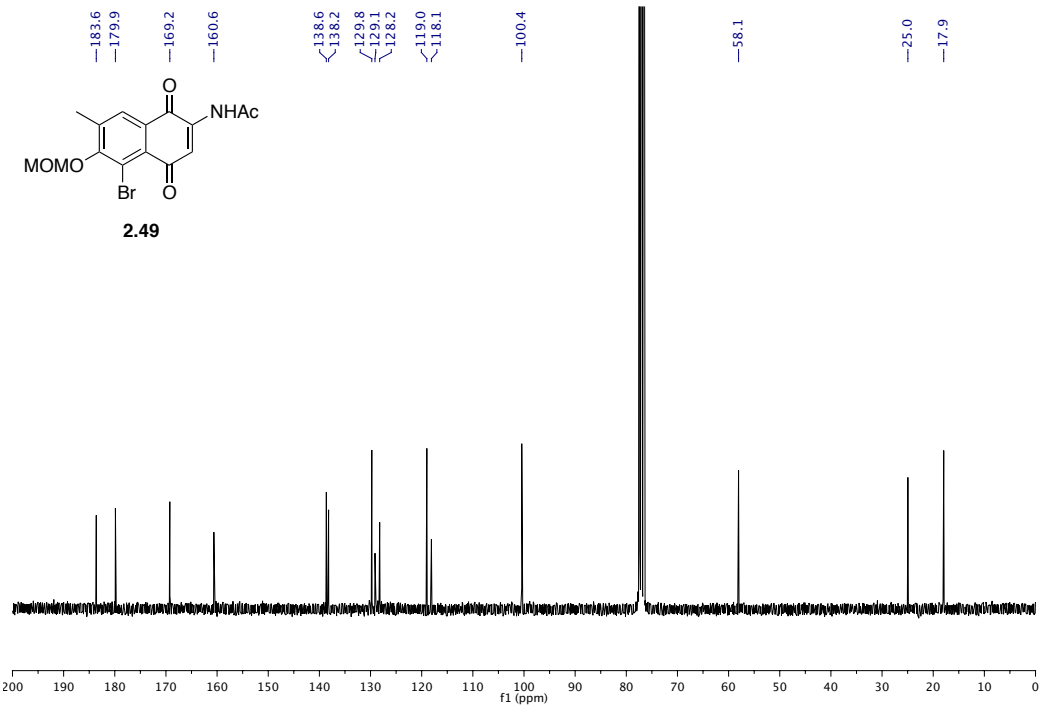
2.47


10.40
9.82
7.89
7.57
2.36
2.21
O
NHAc
HO
Br
O
2.48
1.0
1.1
1.2
1.0
3.2
3.1
f1 (ppm)

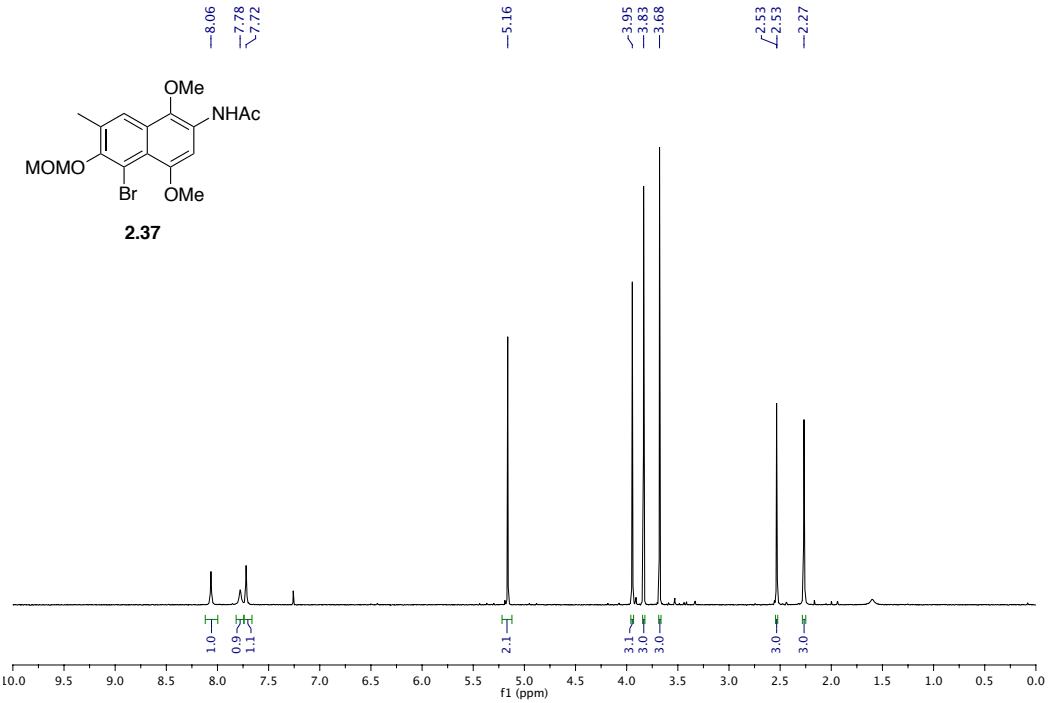

193.3
184.0
178.9
171.2
158.7
139.8
130.8
129.0
128.0
124.4
117.3
24.4
17.3
O
NHAc
HO
Br
O
2.48
f1 (ppm)

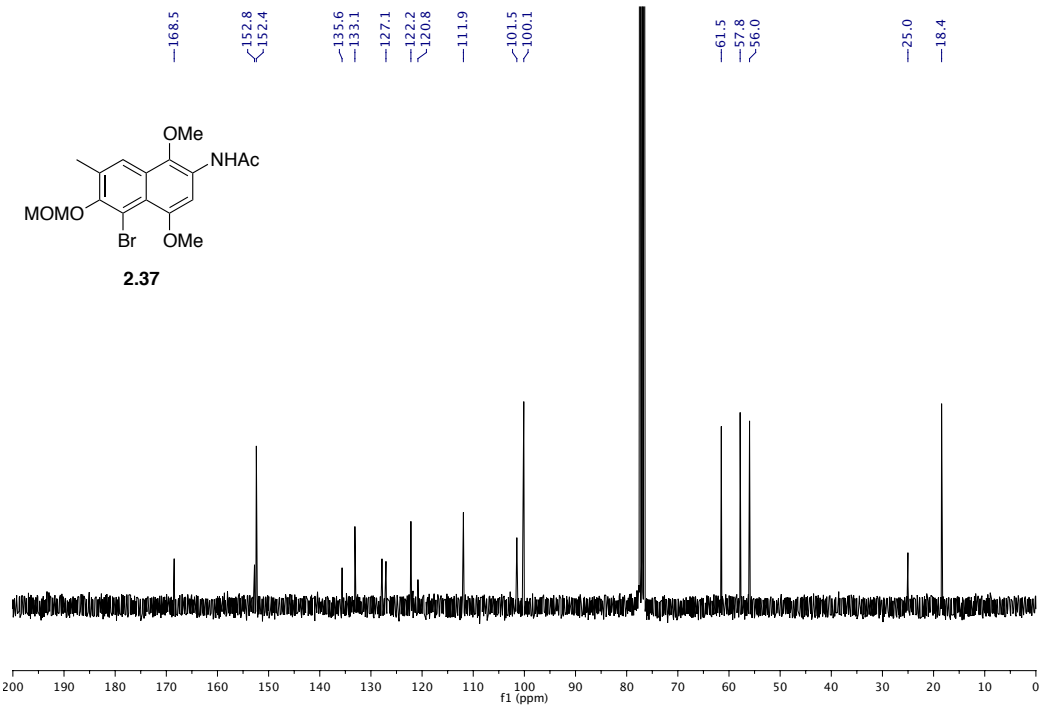

8.22
7.99
7.80
5.18
3.67
2.50
2.50
2.27
O
NHAc
MOMO
Br
O
1.0
1.0
1.0
2.1
3.1
3.0
3.1
f1 (ppm)

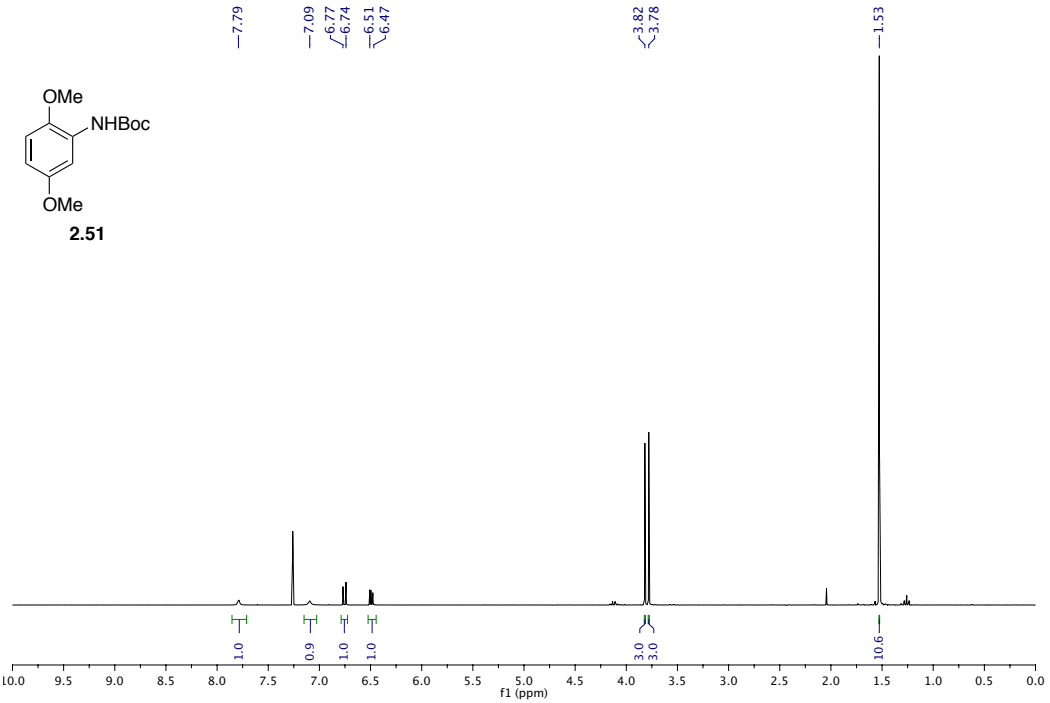
2.49

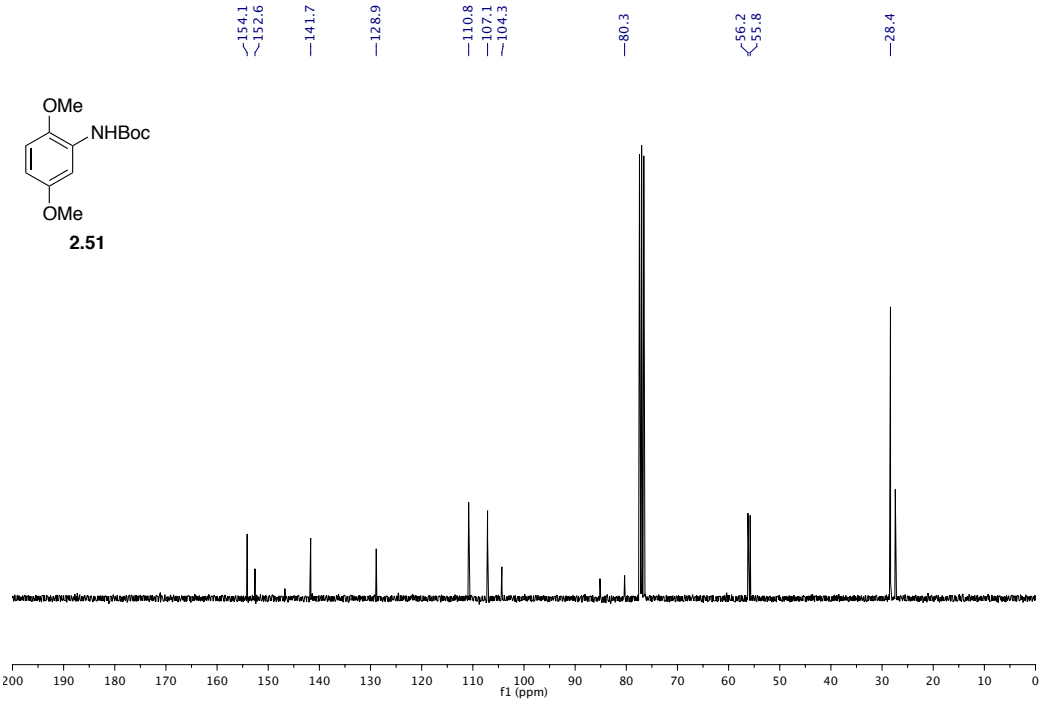

183.6
179.9
169.2
160.6
138.6
138.2
129.8
129.1
128.2
119.0
118.1
100.4
58.1
25.0
17.9
O
NHAc
MOMO
Br
O
f1 (ppm)

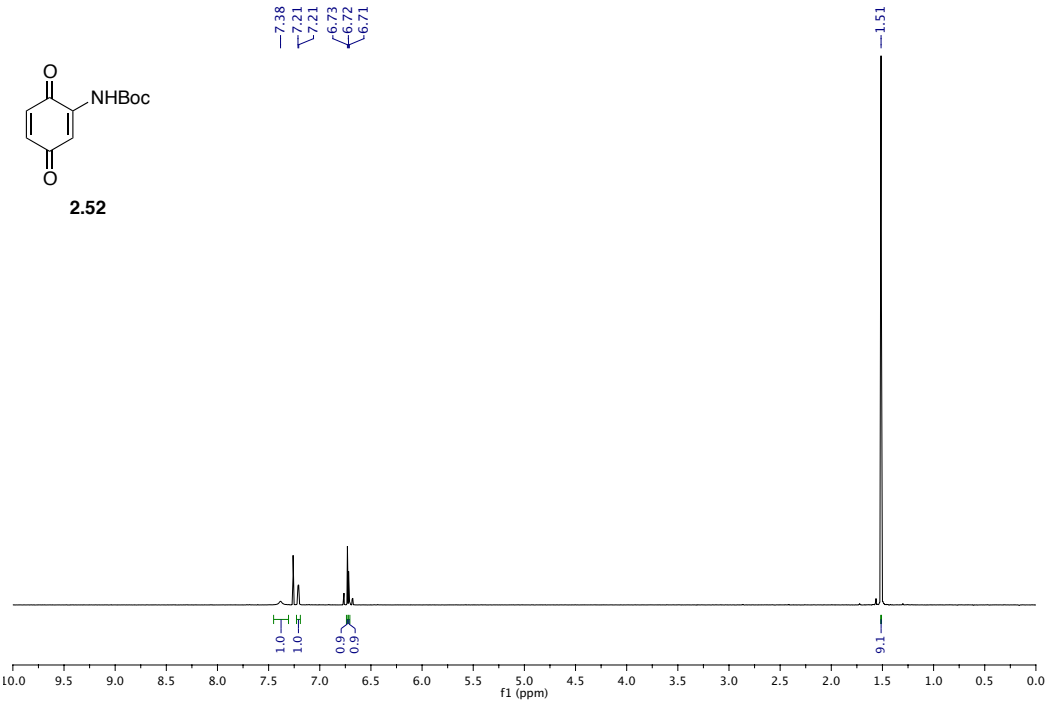
2.49


8.06
7.78
7.72
5.16
3.95
3.83
3.68
2.53
2.53
2.27
OMe
NHAc
MOMO
Br
OMe
2.37
1.0
0.9
1.1
2.1
3.1
3.0
3.0
3.0
3.0
f1 (ppm)

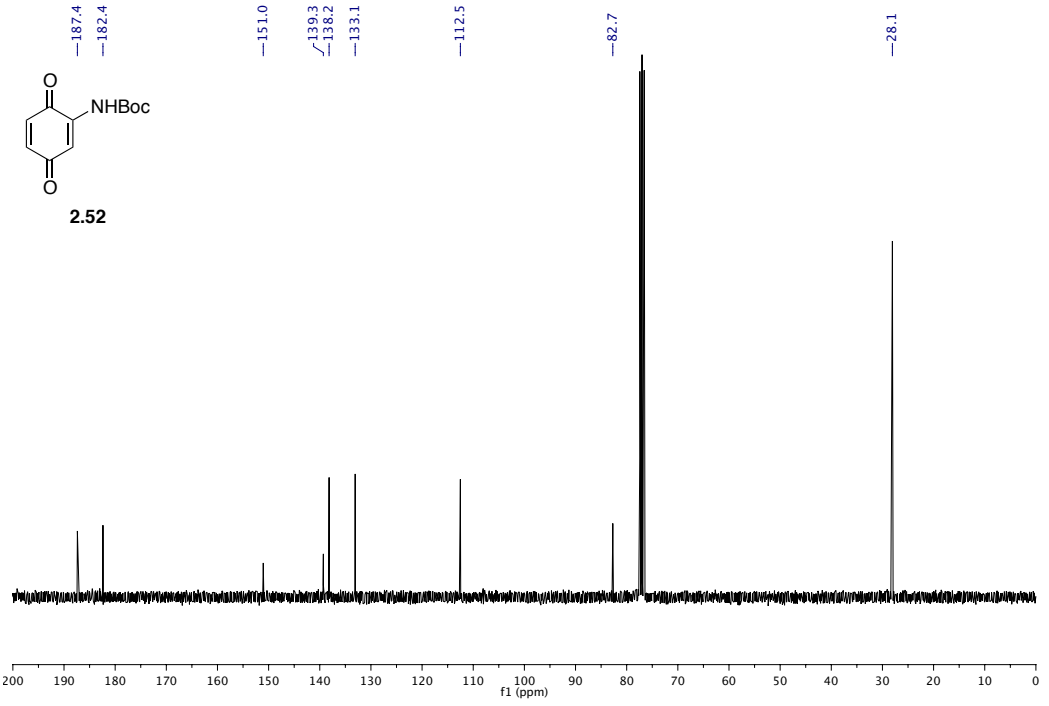

168.5
152.8
152.4
135.6
133.1
127.1
122.2
120.8
111.9
101.5
100.1
61.5
57.8
56.0
25.0
18.4
OMe
NHAc
MOMO
Br
OMe
2.37
f1 (ppm)


7.79
7.09
6.77
6.74
6.51
6.47
3.82
3.78
1.53
OMe
NHBoc
OMe
2.51
1.0
0.9
1.0
1.0
3.0
3.0
10.6
f1 (ppm)

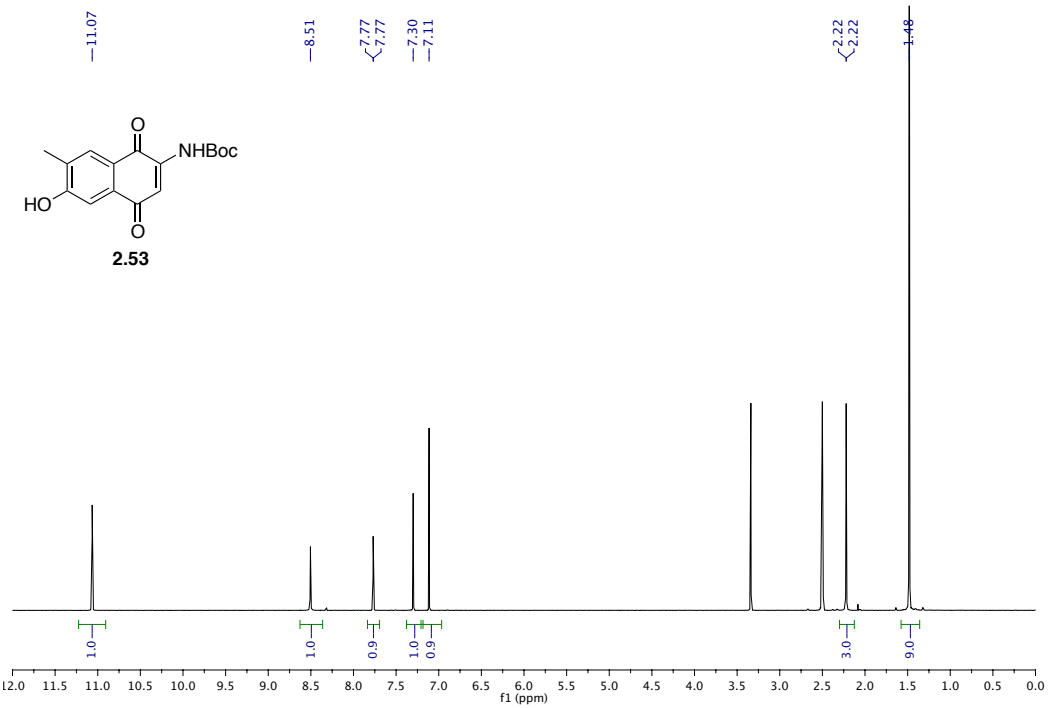

154.1
152.6
141.7
128.9
110.8
107.1
104.3
80.3
56.2
55.8
28.4
OMe
NHBoc
OMe
2.51
f1 (ppm)

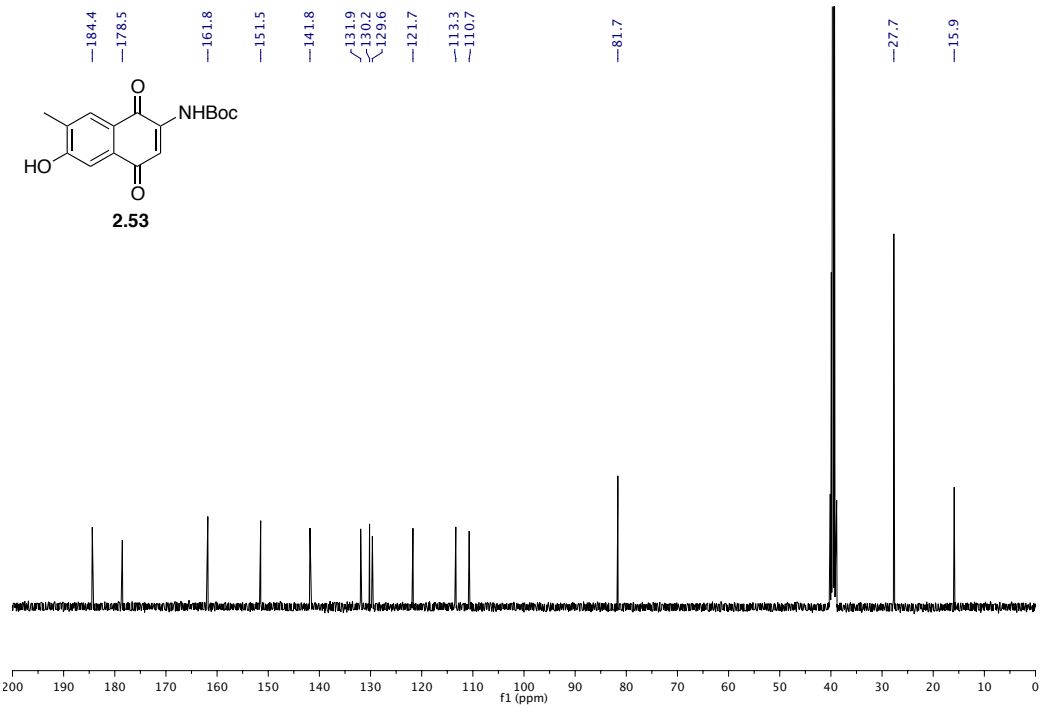

7.38
7.21
7.21
6.73
6.72
6.71
1.51
NHBoc
O
O
1.0
1.0
0.9
0.9
9.1
f1 (ppm)

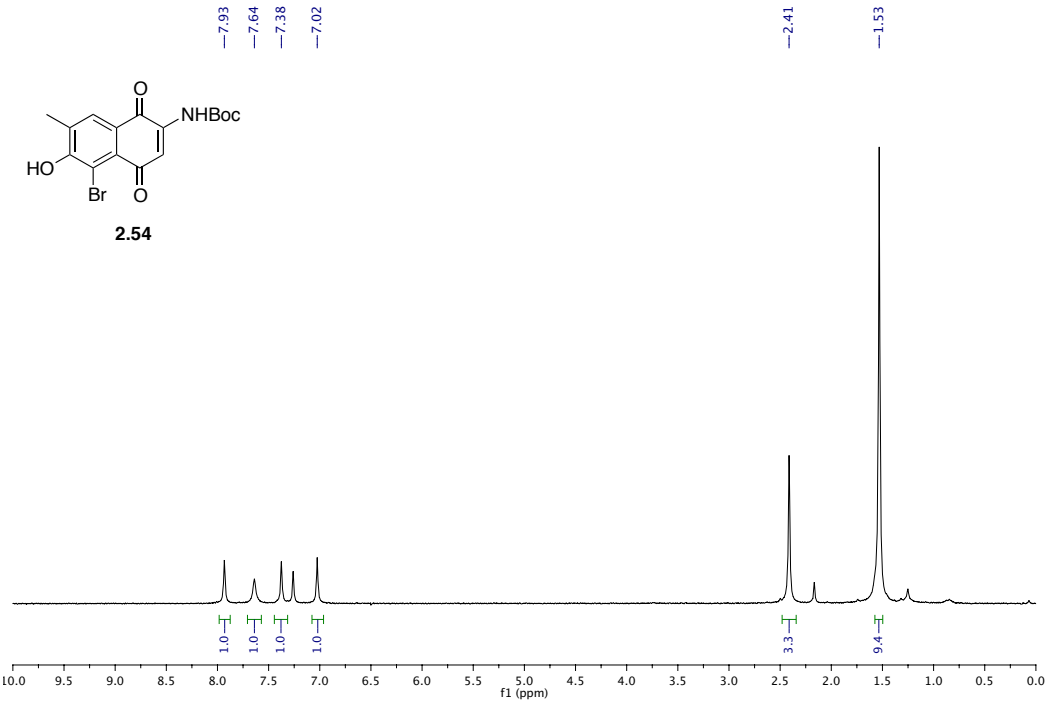
2.52


187.4
182.4
151.0
139.3
138.2
133.1
112.5
82.7
28.1
NHBoc
O
O
f1 (ppm)

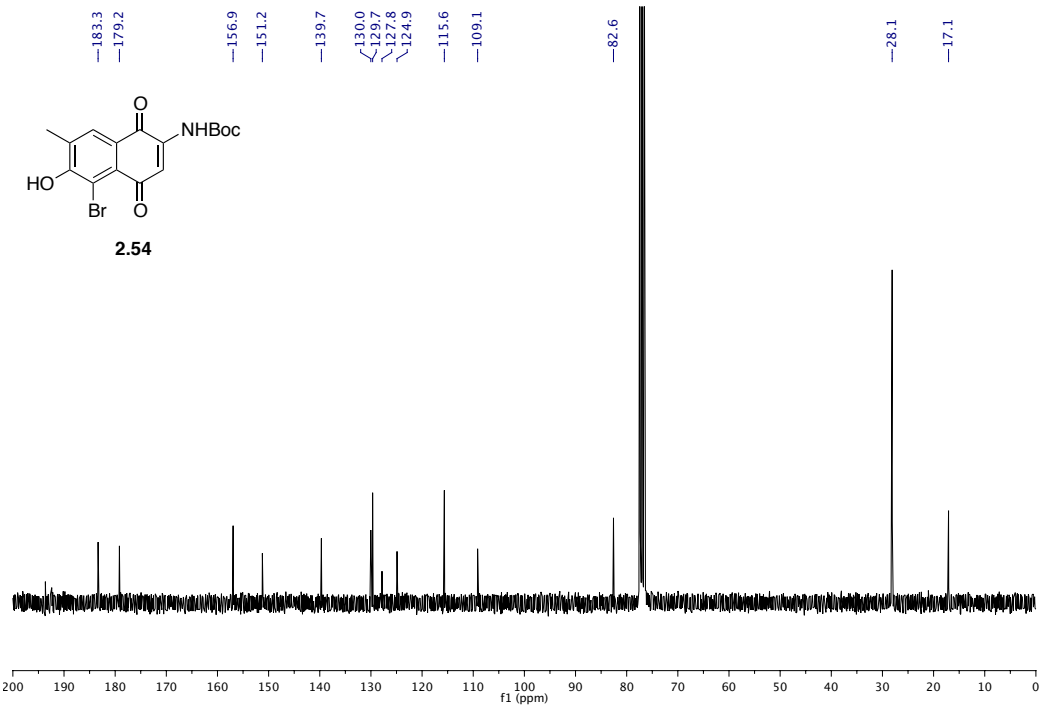
2.52


11.07
8.51
7.77
7.77
7.30
7.11
2.22
2.22
1.48
NHBoc
HO
O
O
2.53
1.0
1.0
0.9
1.0
0.9
3.0
9.0
f1 (ppm)

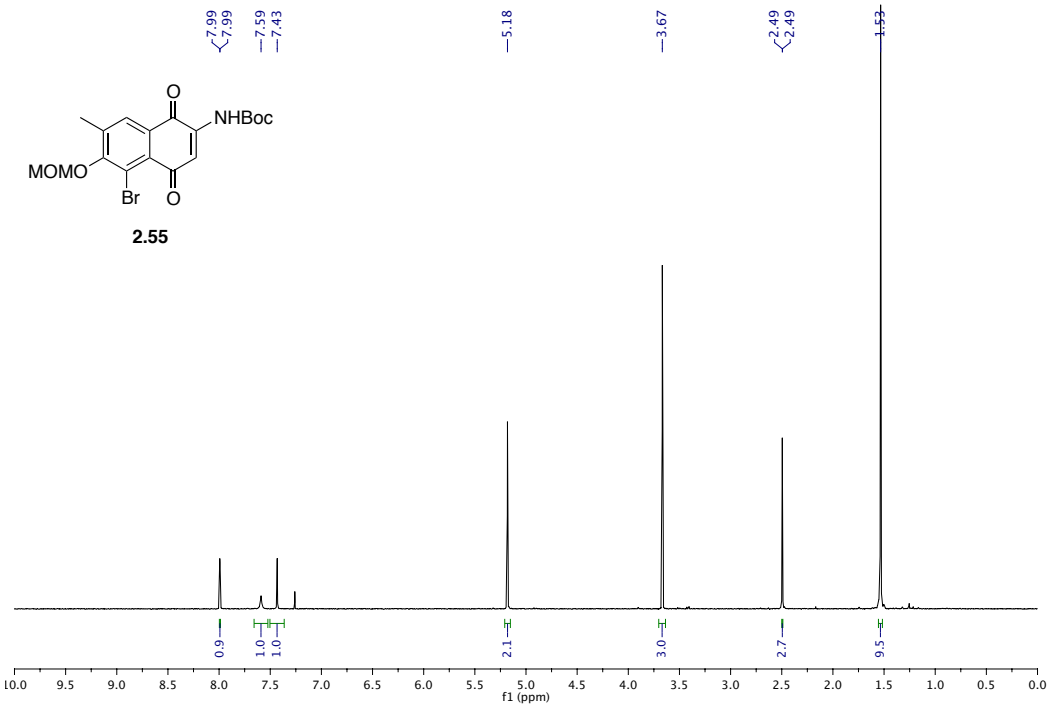

184.4
178.5
161.8
151.5
141.8
131.9
130.2
129.6
121.7
113.3
110.7
81.7
27.7
15.9
NHBoc
HO
O
O
2.53
f1 (ppm)


7.93
7.64
7.38
7.02
2.41
1.53
O
NHBoc
HO
Br
O
1.0
1.0
1.0
1.0
3.3
9.4
f1 (ppm)

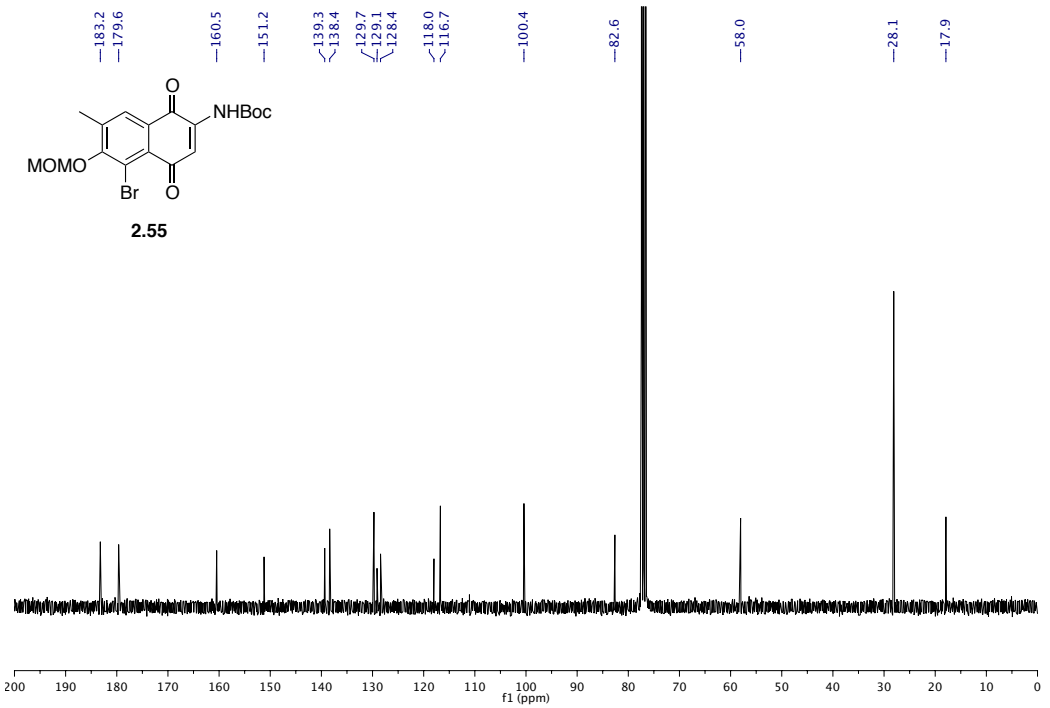
2.54


183.3
179.2
156.9
151.2
139.7
130.0
129.7
127.8
124.9
115.6
109.1
82.6
28.1
17.1
O
NHBoc
HO
Br
O
f1 (ppm)

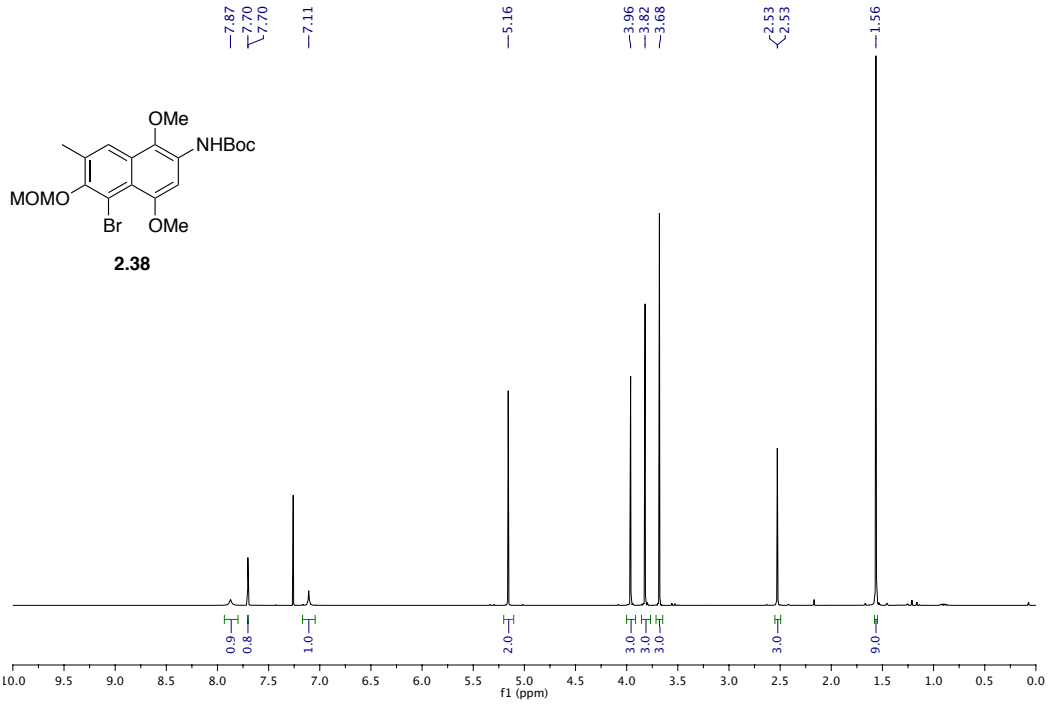
2.54


7.99
7.99
7.59
7.43
5.18
3.67
2.49
2.49
1.53
O
NHBoc
MOMO
Br
O
0.9
1.0
1.0
2.1
3.0
2.7
9.5
f1 (ppm)

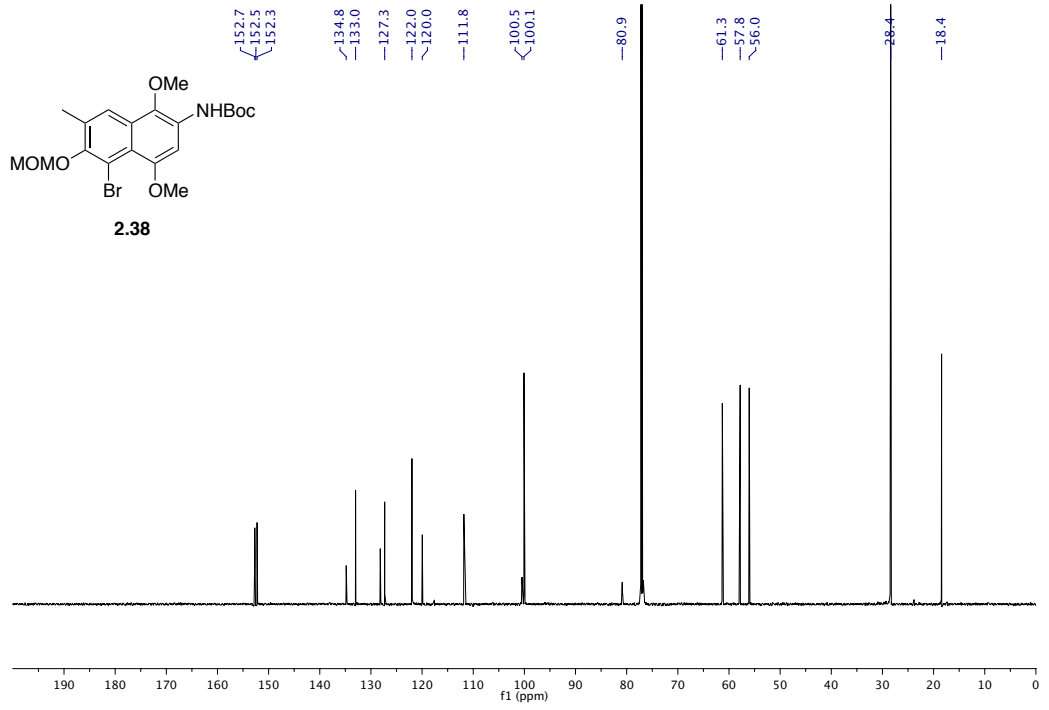
2.55


183.2
179.6
160.5
151.2
139.3
138.4
129.7
129.1
128.4
118.0
116.7
100.4
82.6
58.0
28.1
17.9
O
NHBoc
MOMO
Br
O
f1 (ppm)

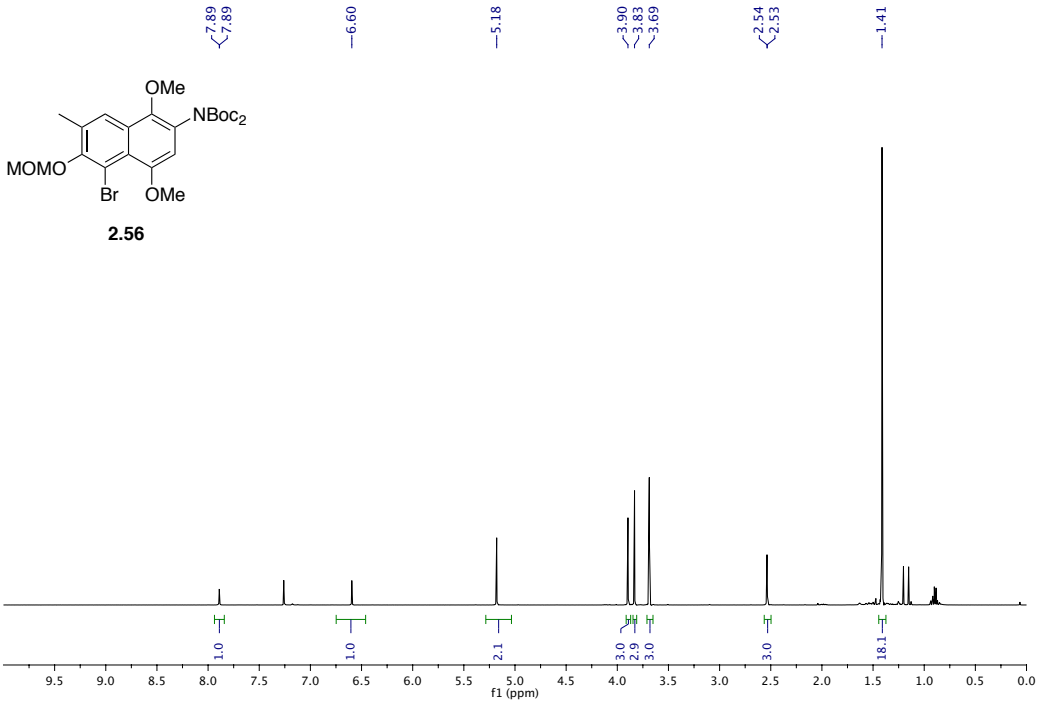
2.55


7.87
7.70
7.70
7.11
5.16
3.96
3.82
3.68
2.53
2.53
1.56
OMe
NHBoc
MOMO
Br
OMe
0.9
0.8
1.0
2.0
3.0
3.0
3.0
3.0
9.0
f1 (ppm)

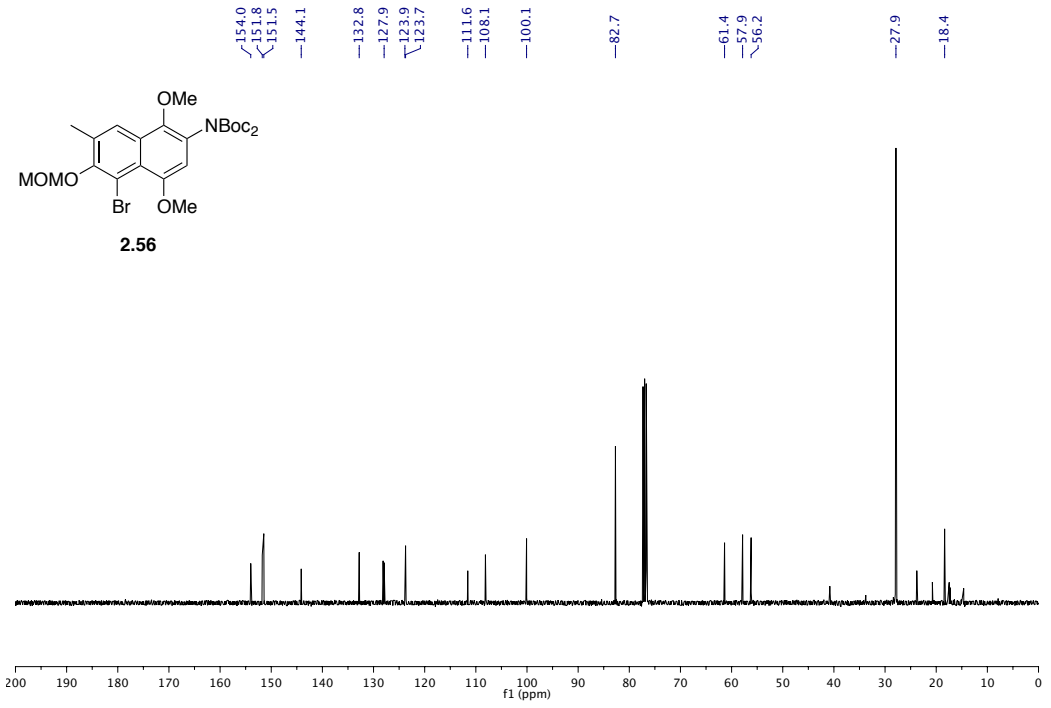
2.38


152.7
152.5
152.3
134.8
133.0
127.3
122.0
120.0
111.8
100.5
100.1
80.9
61.3
57.8
56.0
28.4
18.4
OMe
NHBoc
MOMO
Br
OMe
f1 (ppm)

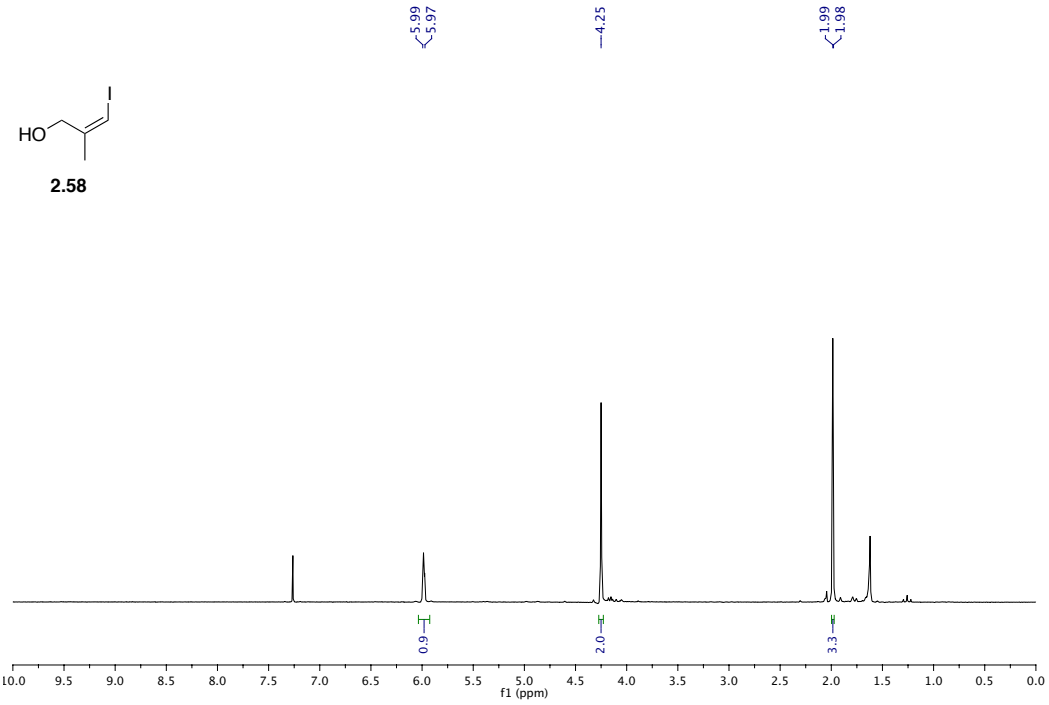
2.38


7.89
7.89
6.60
5.18
3.90
3.83
3.69
2.54
2.53
1.41
OMe
NBoc2
MOMO
Br
OMe
1.0
1.0
2.1
3.0
2.9
3.0
3.0
18.1
f1 (ppm)

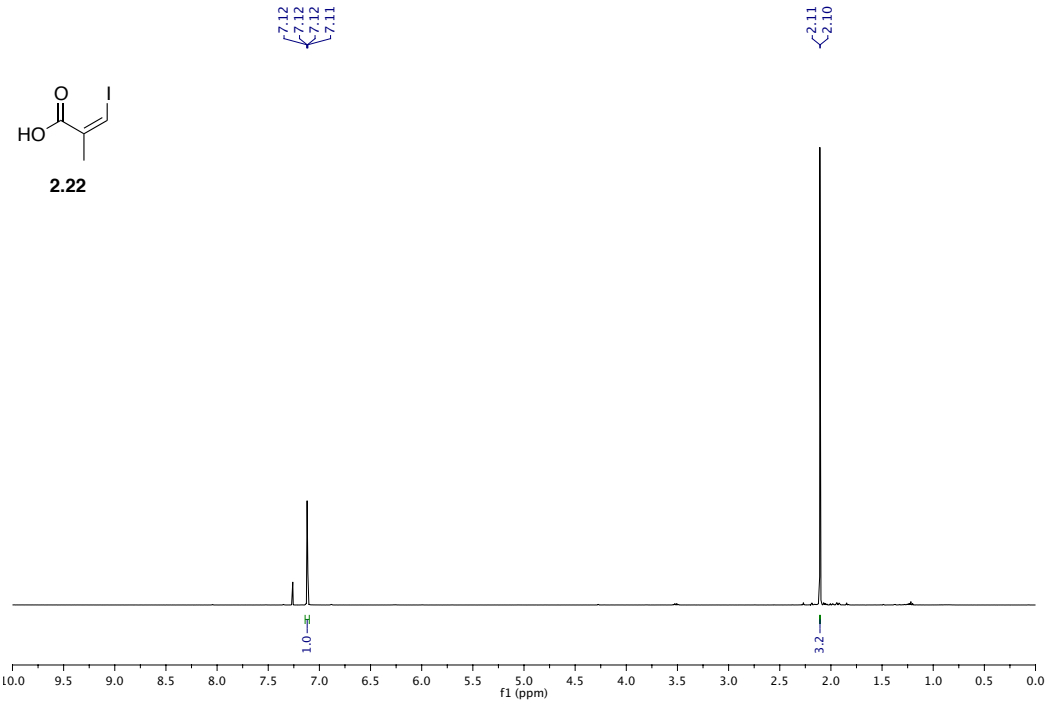
2.56


154.0
151.8
151.5
144.1
132.8
127.9
123.9
123.7
111.6
108.1
100.1
82.7
61.4
57.9
56.2
27.9
18.4
OMe
NBoc2
MOMO
Br
OMe
f1 (ppm)

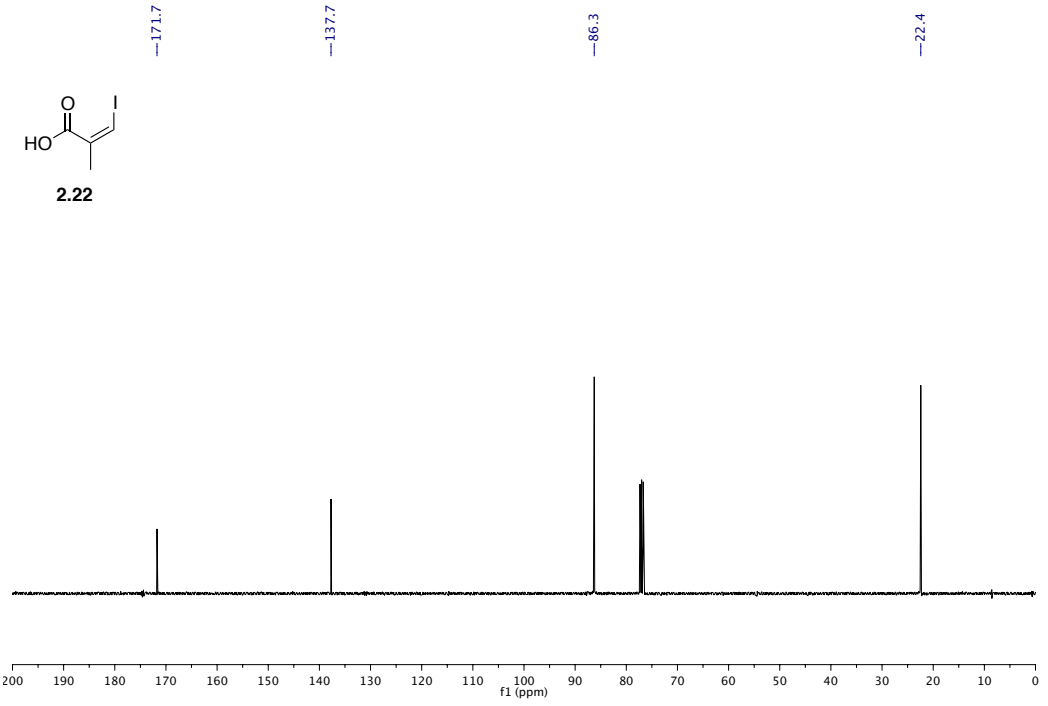
2.56


5.99
5.97
4.25
1.99
1.98
HO
I
0.9
2.0
3.3
10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)

2.58

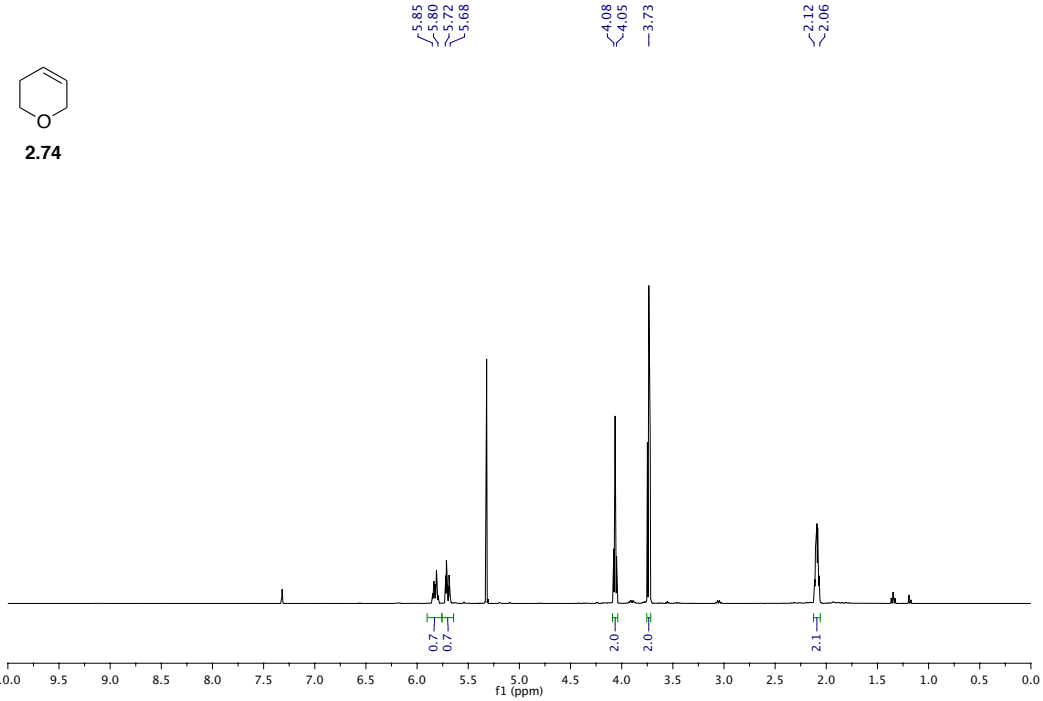

7.12
7.12
7.12
7.11
2.11
2.10
O
I
HO
1.0
3.2
f1 (ppm)

2.22

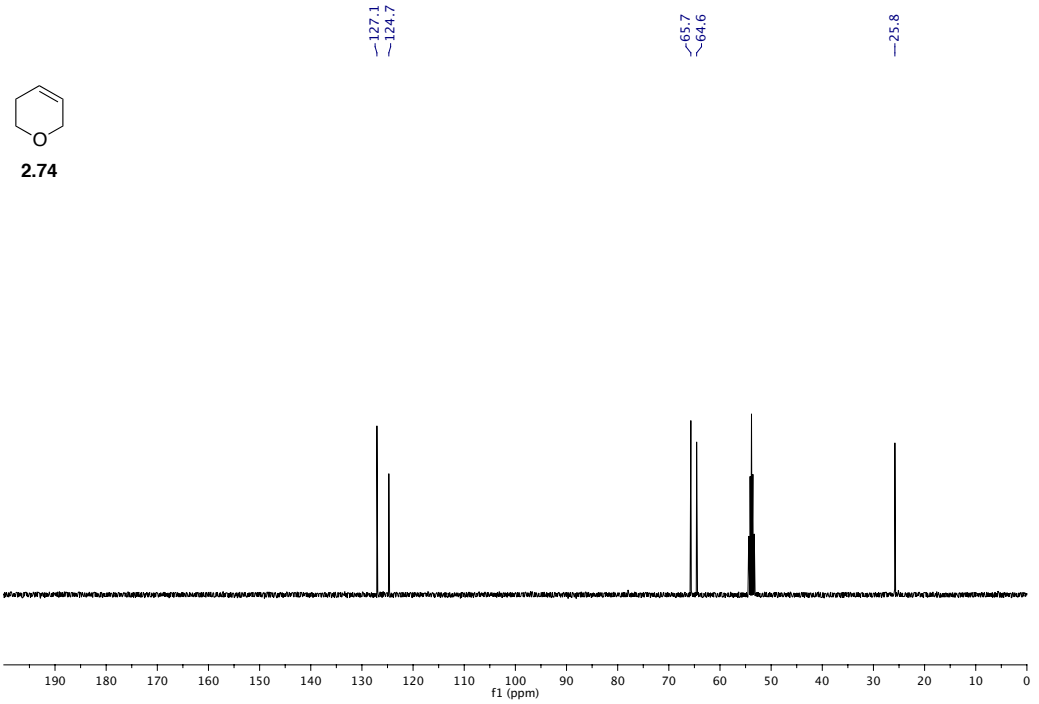

171.7
137.7
86.3
22.4
O
I
HO
f1 (ppm)

2.22

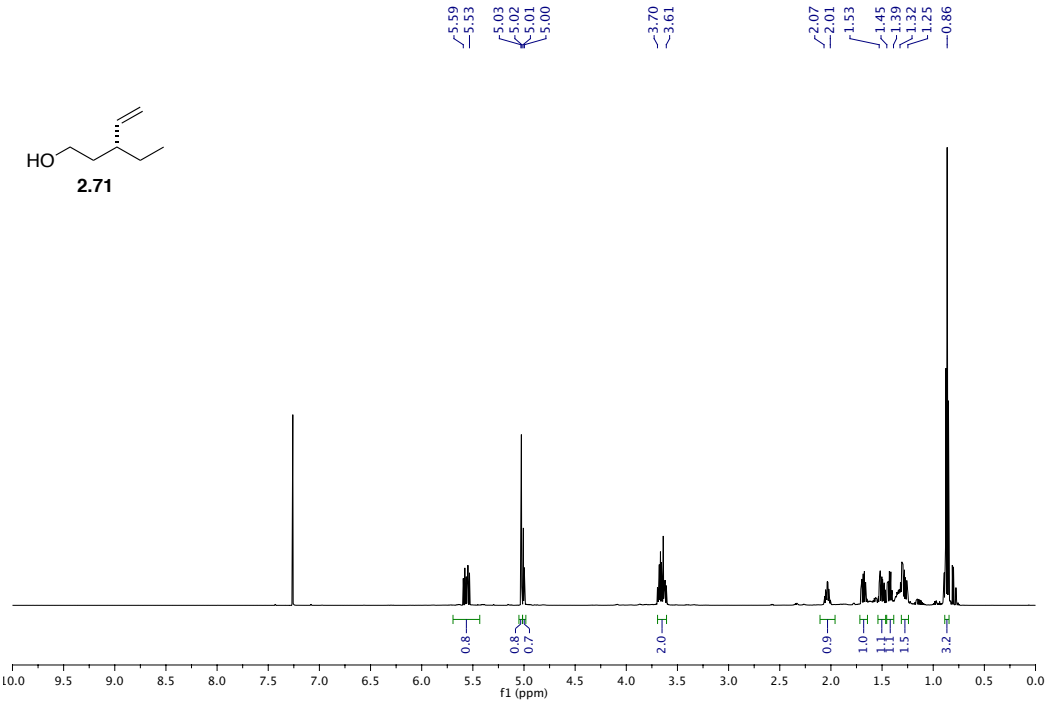
3.4.2 Divergolides C and D Project

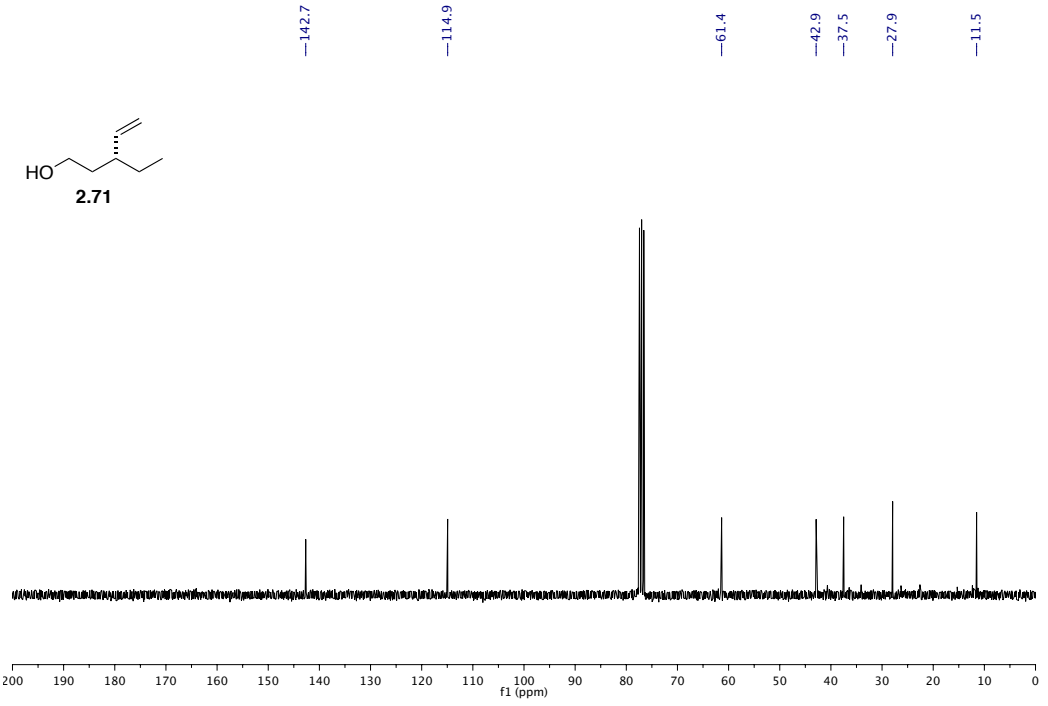


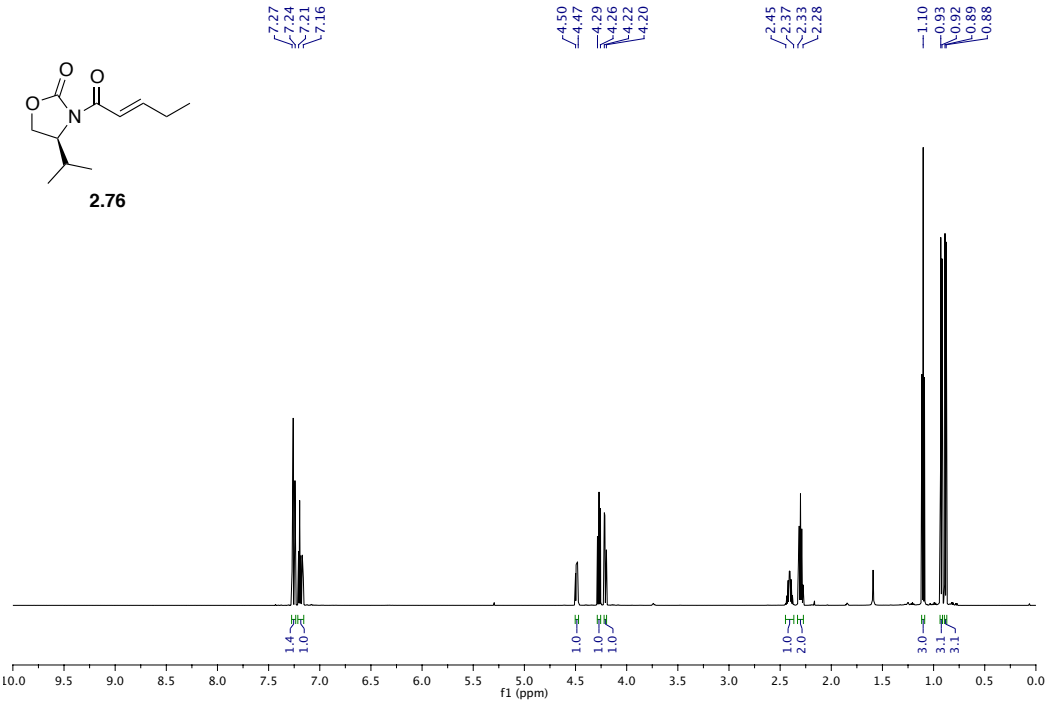
2.74



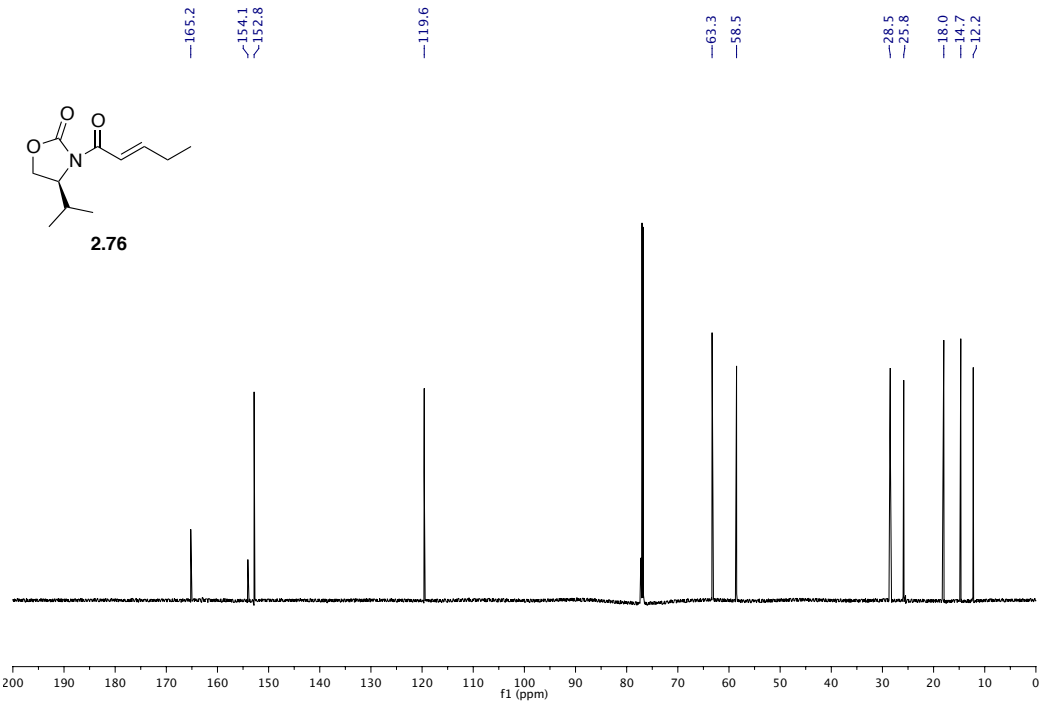
2.74


5.59
5.53
5.03
5.02
5.01
5.00
3.70
3.61
2.07
2.01
1.53
1.45
1.39
1.32
1.25
0.86
HO
2.71
0.8
0.8
0.7
2.0
0.9
1.0
1.1
1.1
1.5
3.2
f1 (ppm)

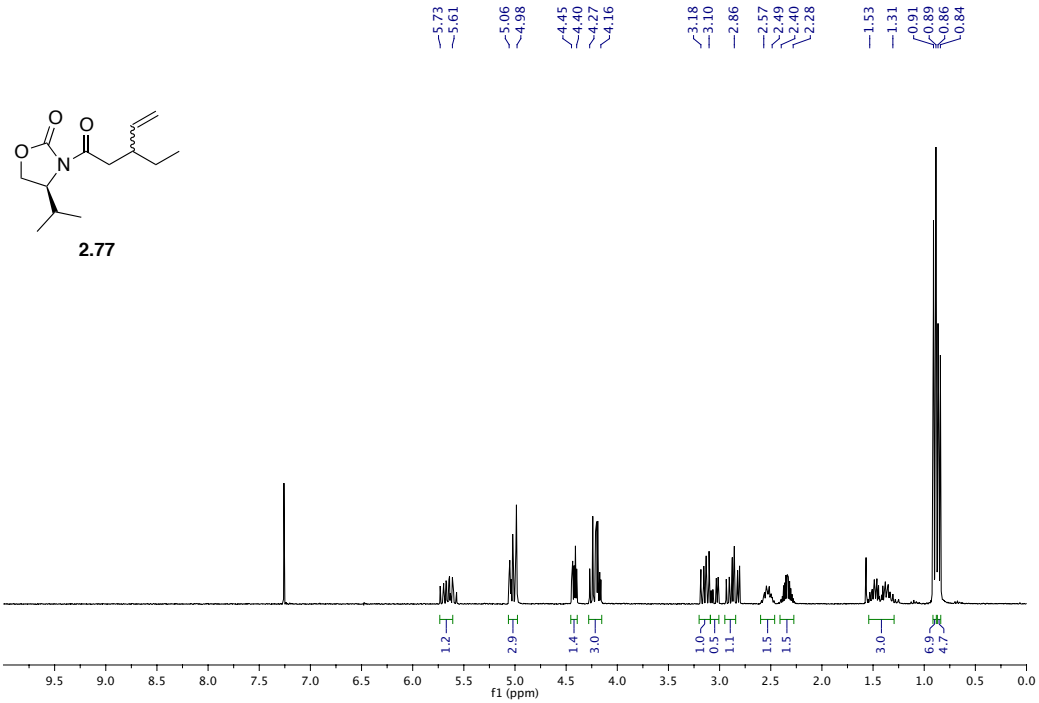

142.7
114.9
61.4
42.9
37.5
27.9
11.5
HO
2.71
f1 (ppm)


7.27
7.24
7.21
7.16
4.50
4.47
4.29
4.26
4.22
4.20
2.45
2.37
2.33
2.28
1.10
0.93
0.92
0.89
0.88
1.4
1.0
1.0
1.0
1.0
1.0
2.0
3.0
3.1
3.1
f1 (ppm)

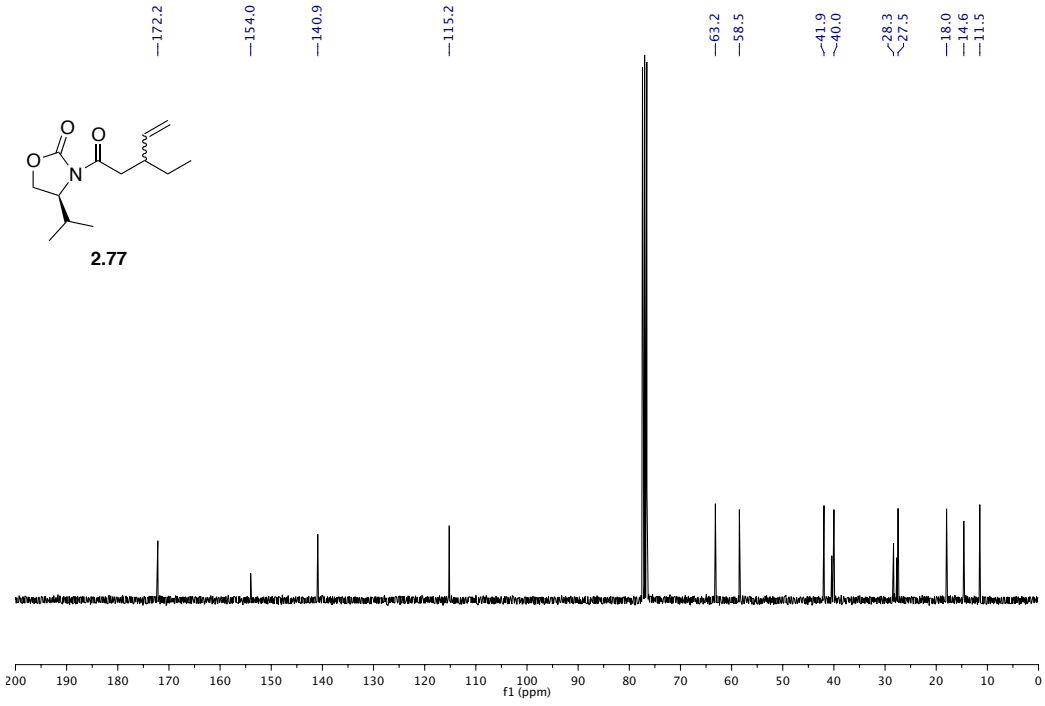
2.76


165.2
154.1
152.8
119.6
63.3
58.5
28.5
25.8
18.0
14.7
12.2
f1 (ppm)

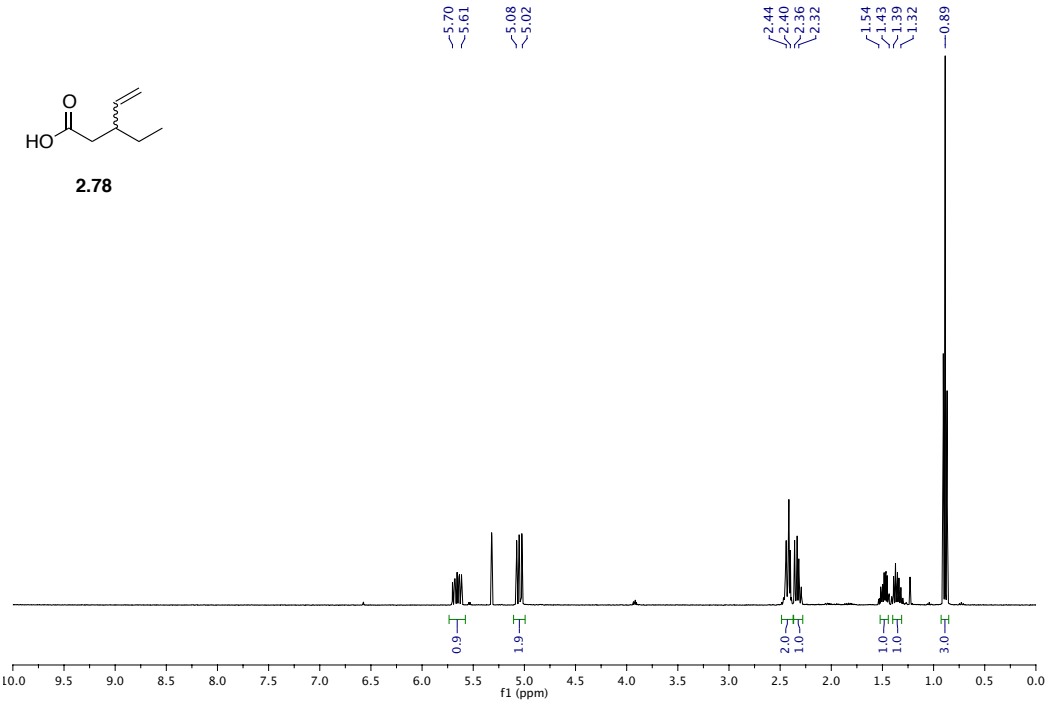
2.76


5.73
5.61
5.06
4.98
4.45
4.40
4.27
4.16
3.18
3.10
2.86
2.57
2.49
2.40
2.28
1.53
1.31
0.91
0.89
0.86
0.84
1.2
2.9
1.4
3.0
1.0
0.5
1.1
1.5
1.5
3.0
6.9
4.7
f1 (ppm)

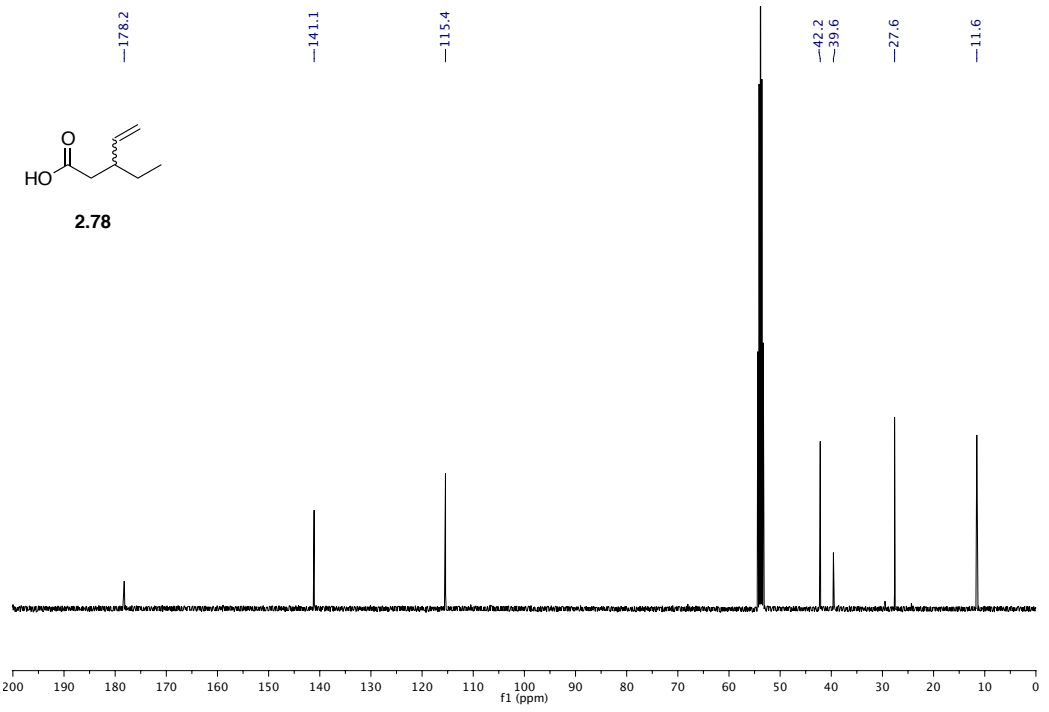
2.77


172.2
154.0
140.9
115.2
63.2
58.5
41.9
40.0
28.3
27.5
18.0
14.6
11.5
f1 (ppm)

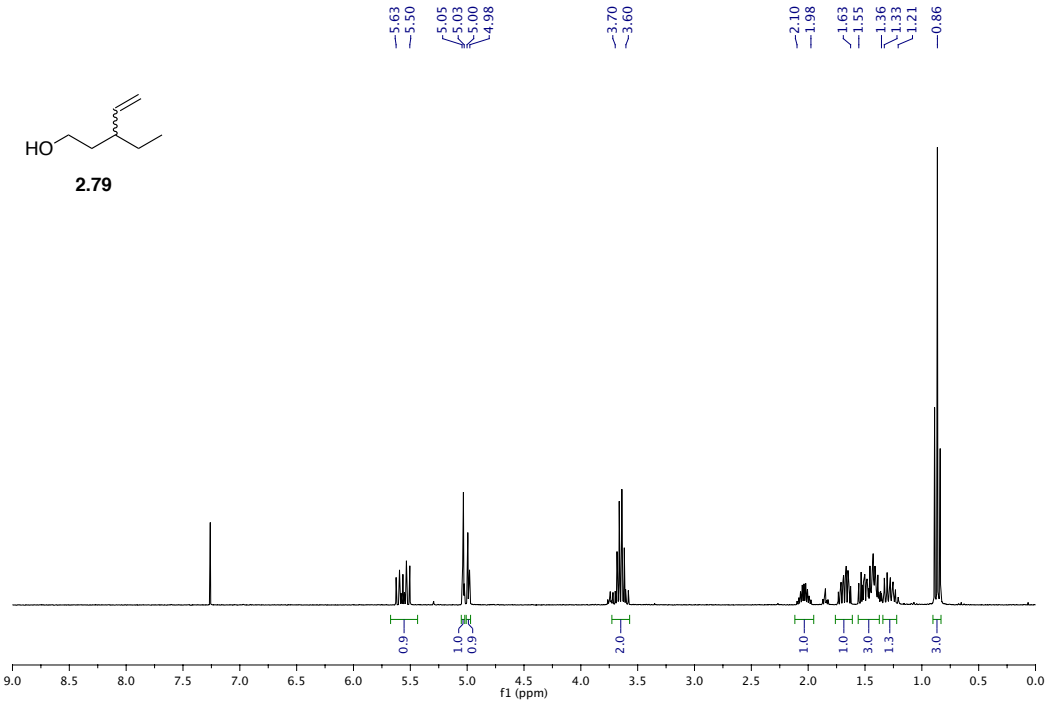
2.77


5.70
5.61
5.08
5.02
2.44
2.40
2.36
2.32
1.54
1.43
1.39
1.32
0.89
O
HO
0.9
1.9
2.0
1.0
1.0
1.0
3.0
f1 (ppm)

2.78


178.2
141.1
115.4
42.2
39.6
27.6
11.6
O
HO
f1 (ppm)

2.78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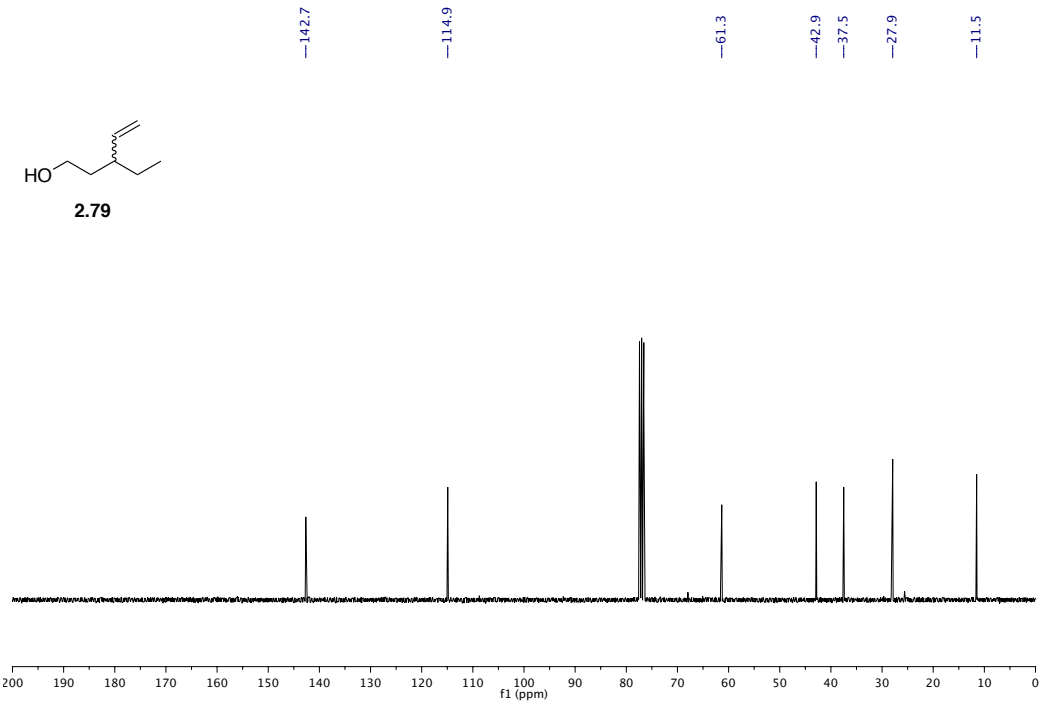
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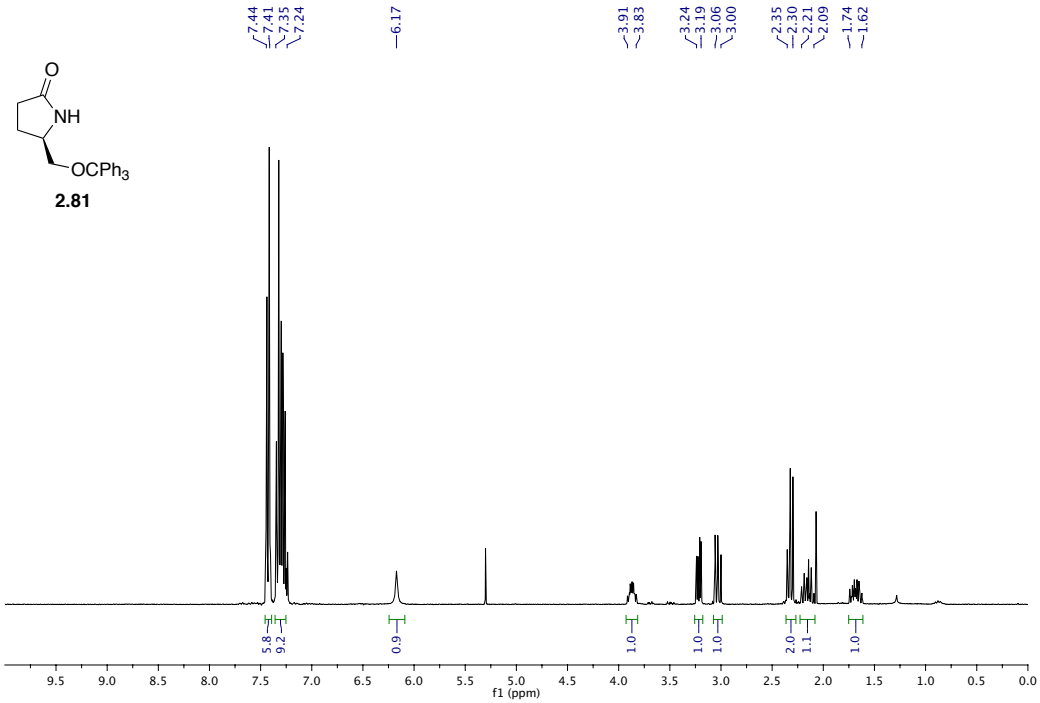
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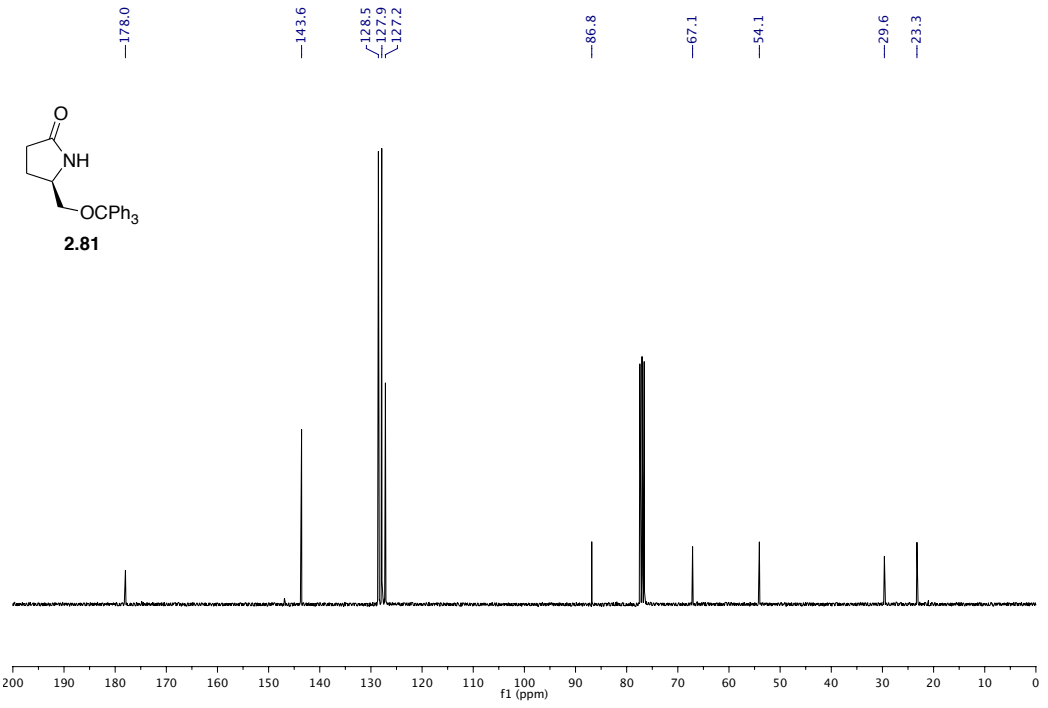
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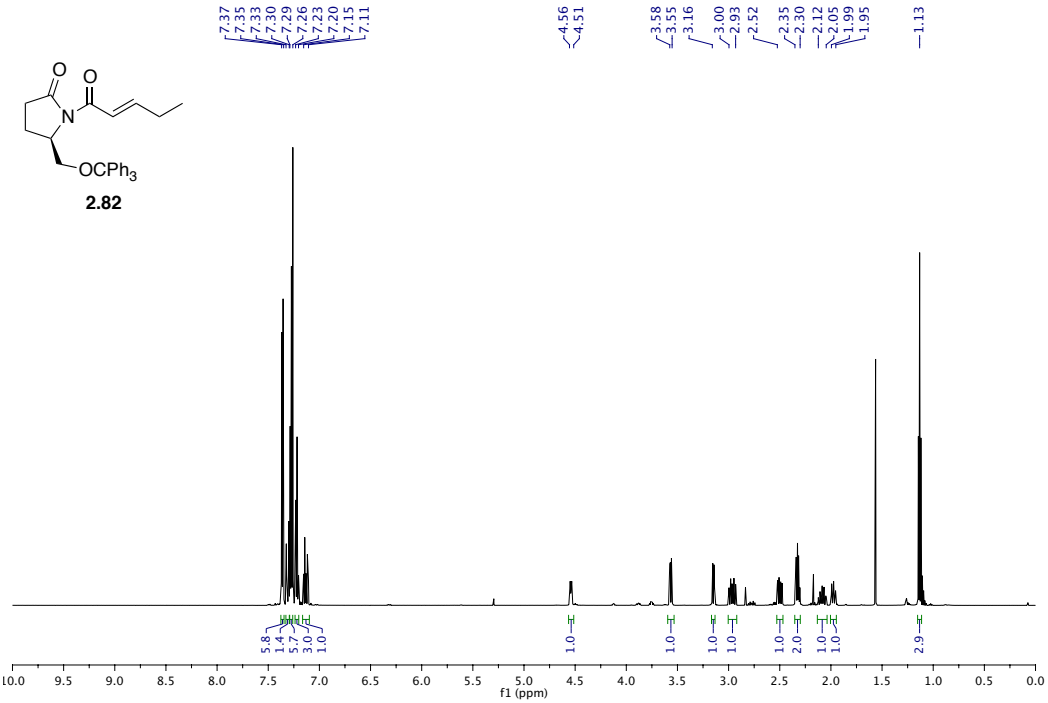
2.81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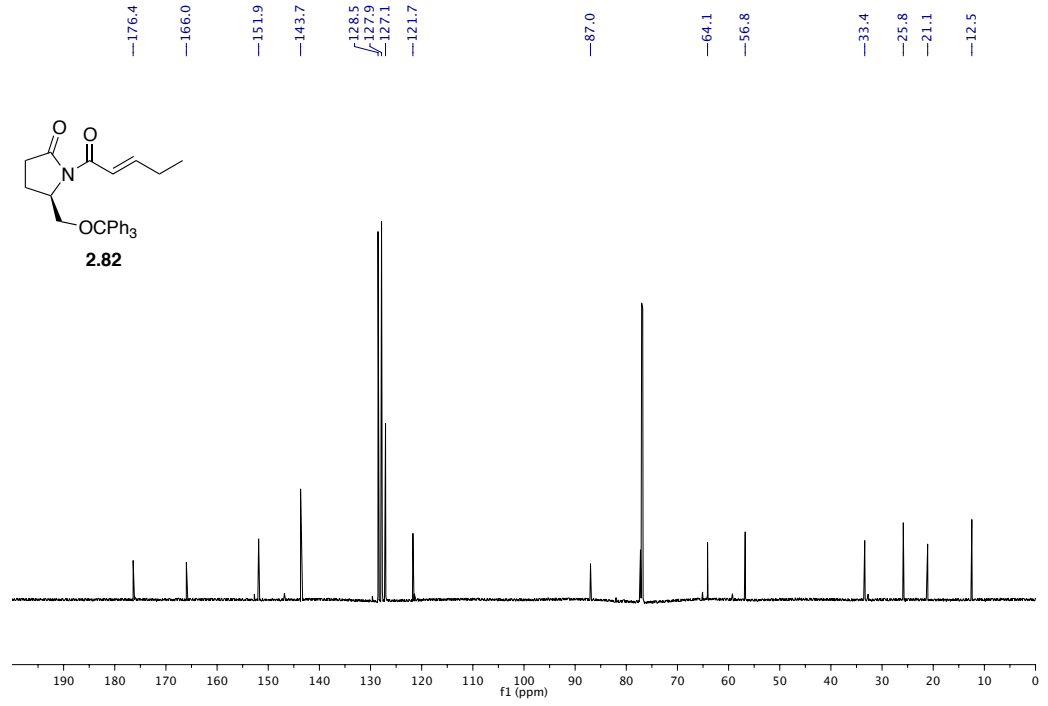
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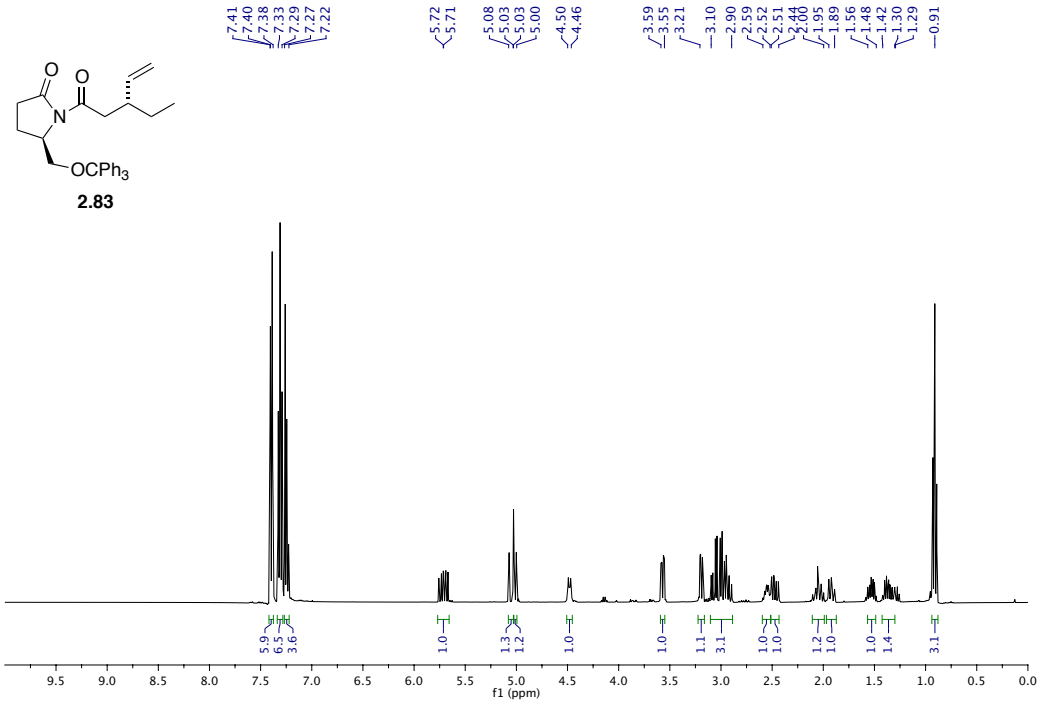
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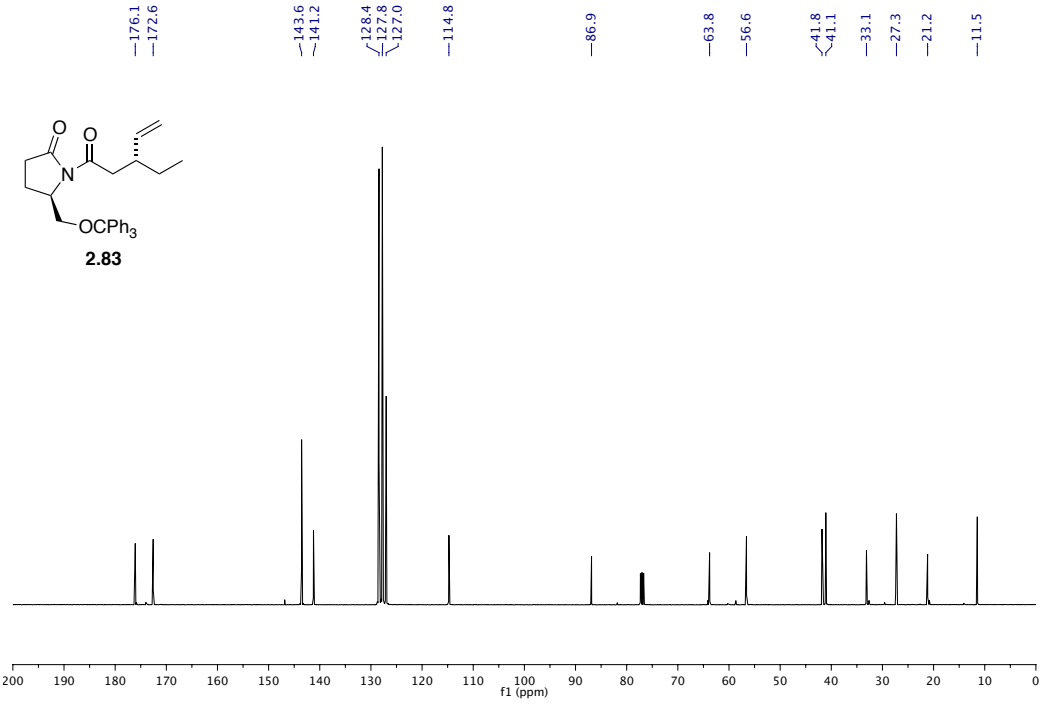
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33.1
27.3
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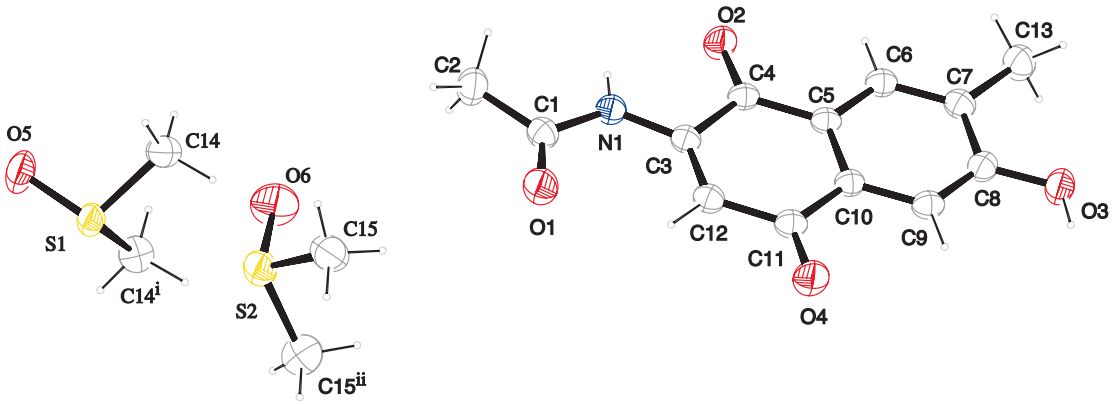
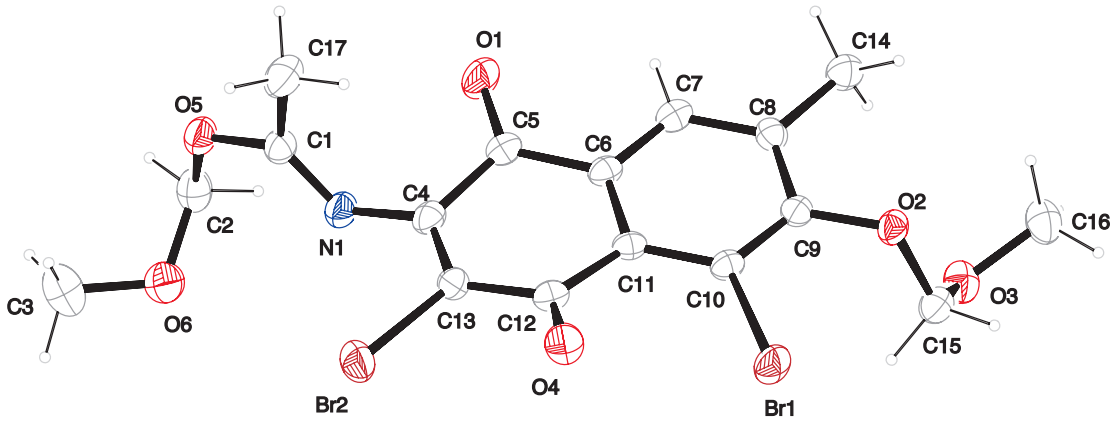
OCPh3
2.83
f1 (ppm)

3.5 Crystal Structures

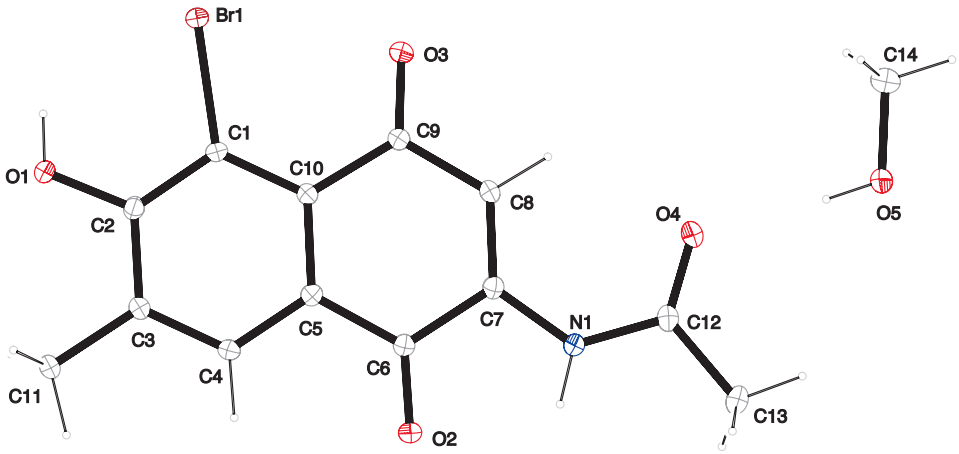
a) X-ray structure of Acetyl-aminonaphthoquinone **2.44**:



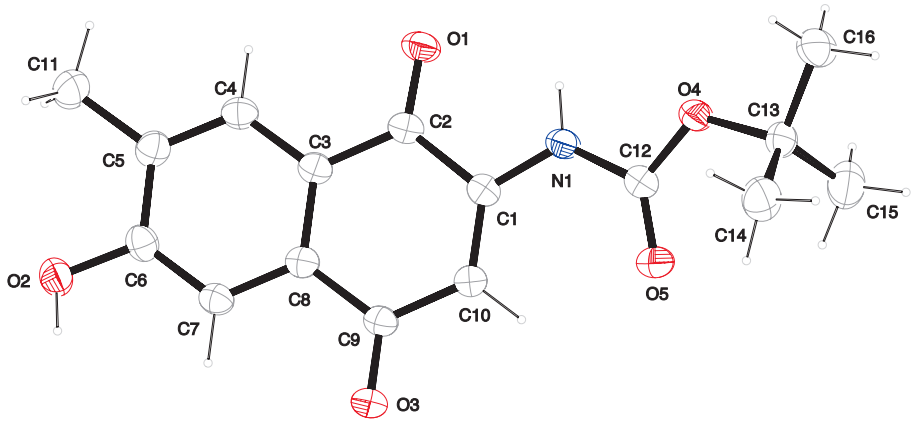
b) X-ray structure of MOM-Dibromo-naphthoquinone **2.47**:



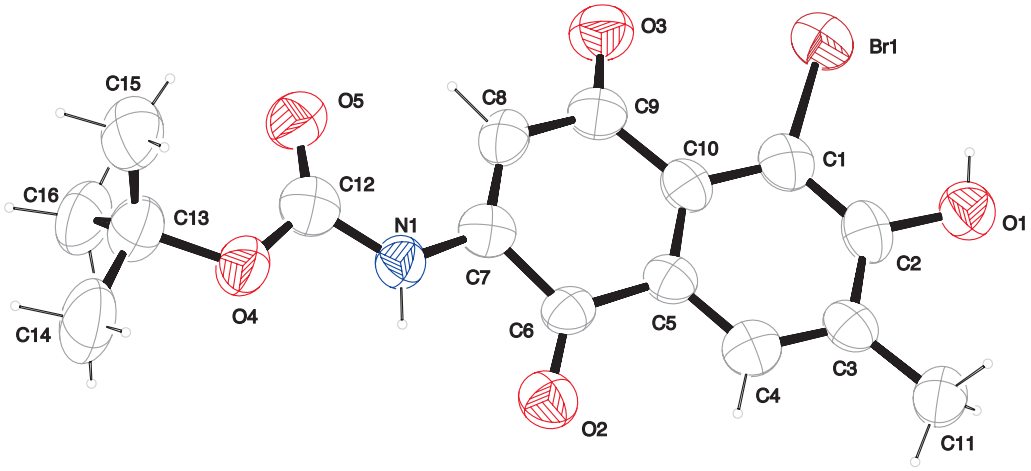
c) X-ray structure of Acetyl-bromonaphthoquinone **2.48**:



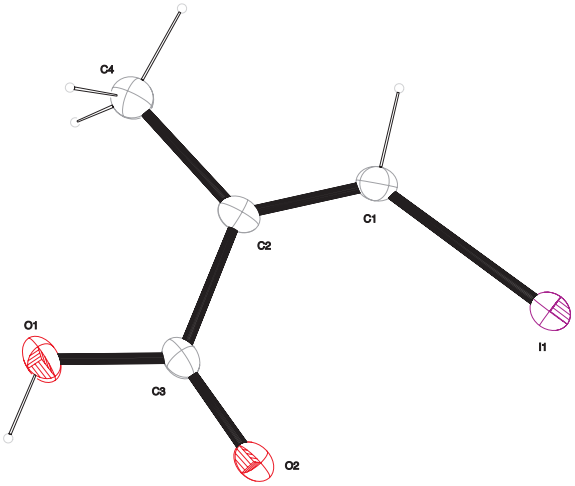
d) X-ray structure of Boc-aminonaphthoquinone **2.53**:



e) X-ray structure of Boc-bromoaminonaphthoquinone **2.54**:



f) X-ray structure of (*Z*)-3-iodo-2-methylacrylic acid (**2.22**):



Part III: Synthesis of Photochromic Open-Channel Blockers

1 Introduction

1.1 Optochemical Genetics

Transmembrane proteins belong to nature´s most important molecular devices. They not only control the transport of specific substances into or out of a cell, but they also allow a cell to communicate with its environment, rendering it susceptible to a variety of input signals. As such, the ability to control transmembrane proteins could aid understanding and helping to cure diseases that are caused by a mal- or disfunction of these proteins. In recent years, a groundbreaking new approach towards this goal has emerged, called "optochemical genetics".[1] In essence, this method utilizes artificial photoreceptors or light-sensitive small molecules to control protein function or neuronal activity. Amongst the vast number of different methods that are used to render receptors light-sensitive, three strategies involving synthetic small molecules have proven particularly useful in our own research (Figure 1).[1a] The simplest and oldest strategy utilizes caged ligands (CLs) (Figure 1, A). These are molecules that bear a functional group which is essential for the ligand-receptor interaction, but masked with a photolabile protecting group. Upon irradiation, the protecting group is cleaved and the active ligand can trigger its biological action. Although CLs have been used in neuroscience, they suffer from several disadvantages. Besides potential toxicity issues with byproducts arising from the uncaging process (i.e. remnants of the protecting group) and off-target effects, there is no more external control possible once the caged ligand has been set free. Thus, a second, so called photochromic ligand (PCL) approach (Figure 1, B) has emerged that is able to overcome some of the latter drawbacks. It employs photoswitchable molecules that can change their configuration when irradiated with light of a certain wavelength. This configurational change is reversible and since the ligand is typically only active in one configuration, the biological effect of the photoswitchable ligand can be switched "on" or "off" using light of a certain wavelength. PCLs can be, like other small molecule drugs, easily applied and they distribute quickly in tissues. However, if a high

[1] (a) Fehrentz, T.; Schönberger, M.; Trauner, D. *Angew. Chem. Int. Ed.* **2011**, *50*, 12156–12182; (b) Deisseroth, K. *Nat. Methods* **2011**, *8*, 26–29; (c) Peron, S.; Svoboda, K. *Nat. Methods* **2011**, *8*, 30–34; (d) Sjulson, L.; Miesenböck, G. *Chem. Rev.* **2008**, *108*, 1588–1602; (e) Zhang, F.; Aravanis, A. M.; Adamantidis, A.; de Lecea, L.; Deisseroth, K. *Nat. Rev. Neurosci.* **2007**, *8*, 577–581.

selectivity for a certain receptor subtype and cellular targeting is desired, a third approach, named photoswitchable tethered ligand (PTL) approach (Figure 1, C) comes into play. In this scenario, the photoswitchable ligand carries a reactive group that allows it to be tethered to the target receptor by means of bioconjugation. Since the point of attachment can be a cysteine or another reactive amino acid side chain, the PTLs can be genetically encoded. Similar to the PCLs, PTLs can be reversibly switched and thus allow for the controlled triggering of a biological response.

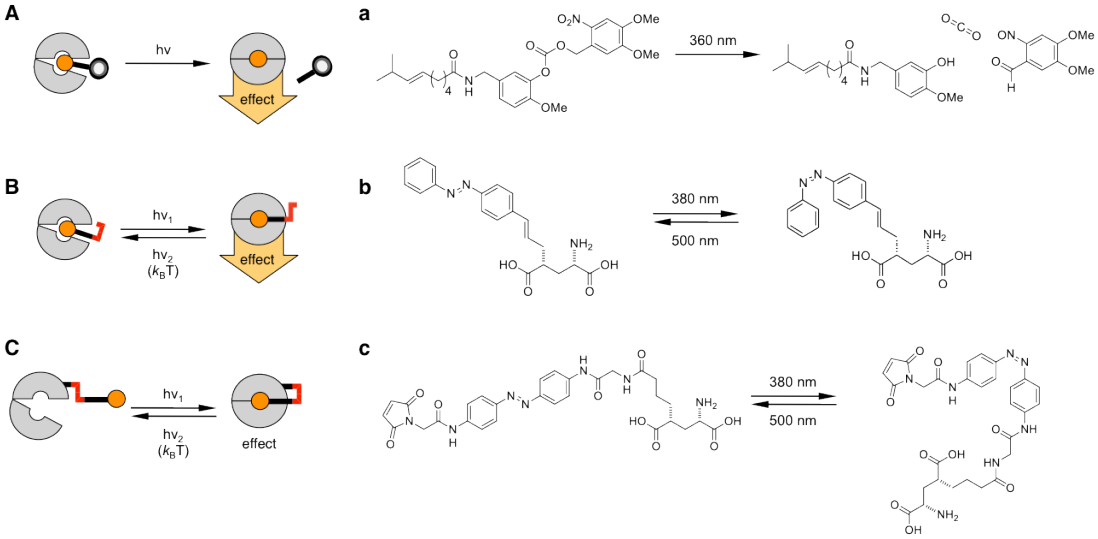


Figure 1. Three approaches, that provide the basis for optochemical genetics are shown. A) Upon irradiation, the caged ligand (CL) breaks apart and is converted into its active form. a) Capsaicin, once uncaged, can stimulate TRPV1 channels. B) Photochromic ligands (PCLs) can be reversibly converted into their active or inactive state by light. b) 4-GluAzo as an example for a PCL. C) A photoswitchable, thethered ligand (PTL) can selectively be attached to a target through bioconjugation and subsequently be activated by light irradiation. c) MAG-1 is a typical PTL bearing a maleimide group as a site of attachment.[1a]

1.2 The Azobenzene Scaffold

Azobenzenes have turned out to be an ideal platform for the development of PTLs and PCLs in our group.[2] This can be attributed to unique features of the azobenzene scaffold. As such, azobenzenes can be switched from *cis* to *trans* and vice versa when irradiated with light of a certain wavelength. This leads to a change in configuration and length and can be used to substantially alter the distance between substituents on the aromatic core (Figure 2). They can be photoisomerized with light of relatively low intensity since they have high extinction coefficients and quantum yields. Their photostability can be attributed to the fact that switching of azobenzenes occurs at high rates which prevents intersystem crossing and the formation of triplet diradicals, that can in turn react with triplet oxygen to form highly reactive and cytotoxic singlet oxygen. Finally, given the rich and established chemistry of

[2] Banghart, M. R.; Volgraf, M.; Trauner, D. *Biochemistry* **2006**, *45*, 15129–15141.

azobenzenes, they can be easily synthesized[3] and almost all of their properties can be tuned by selective functionalization of their core.

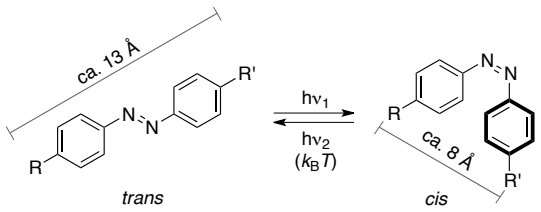


Figure 2. Depending on their substitution, azobenzenes can be switched from a *trans*- to a *cis*-form by irradiation with light of different wavelengths. This *cis*-form can revert thermally or photochemically to the usually more thermodynamically stable *trans*-form.

1.3 QAQ

In recent years, a large number of powerful PTLs and PCLs have been synthesized in our group and subsequently used to address fundamental questions in neurobiology. The most recently developed PCL was QAQ (quarternary ammonium-azobenzene-quaternary-ammonium, **3.3**), a photoswitch that allows for the optical control of nociception through regulation of voltage-gated Na^+, Ca^{2+} and K^+ channels.[4] To date, the study of nociceptive neurons, called nociceptors, has been hampered by the fact that they are inaccessible to selective electrophysiological manipulation due to their small central synaptic terminals and peripheral sensory endings. Selective silencing of nociceptors was first achieved by delivery of QX-314 (**3.2**)[5], a membrane-impermeant derivative of the local anesthetic lidocaine (**3.1**). However, silencing caused by **3.2** is not reversible, which precludes its usefulness for the temporally precise control of nociception. To overcome this limitation, QAQ (**3.3**) was developed (Figure 3). This azobenzene, which is flanked on both ends by a quaternary ammonium group, can be switched from its elongated *trans*- to its bent *cis*-form when irradiated with 380-nm light. This configurational change spontaneously reverses in the dark or can be triggered in milliseconds upon illumination with 500-nm light. It has been shown that QAQ is a potent blocker of voltage-gated ion channels in its *trans*-form, but not in its *cis*-form. It allows for reversible optical silencing of nociceptor activity and was shown to act as a light-sensitive analgesic on the cornea of rats, *in vivo*.

[3] Hamon, F.; Djedaini-Pilard, F.; Barbot, F.; Len, C. *Tetrahedron* **2009**, *65*, 10105–10123.
[4] Mourot, A.; Fehrentz, T.; Le Feuvre, Y.; Smith, C. M.; Herold, C.; Dalkara, D.; Nagy, F.; Trauner, D.; Kramer, R. H. *Nat. Methods* **2012**, *9*, 396–402.
[5] Binshtok, A. M.; Bean, B. P.; Woolf, C. J. *Nature* **2007**, *449*, 607–610.

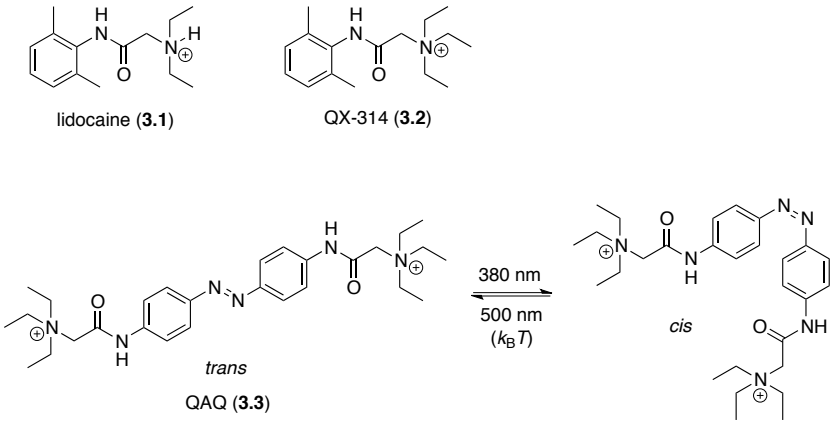


Figure 3. Chemical structures of lidocaine (**3.1**), QX-314 (**3.2**) and QAQ (**3.3**).

Although QAQ has turned out to be a powerful tool for investigating nociceptors, its application could be limited by the fact that switching of *trans*-QAQ to *cis*-QAQ requires UV-light. The latter is not only harmful to biological systems but also unable to penetrate deeper into tissue. We thus targeted QAQ derivatives, which undergo photoswitching upon irradiation with longer wavelengths, but still retain their desirable features in altering channel activity. Our results towards this goal are presented in the following section.

2 Results

2.1 Published Results

2.1.1 Exploring the Pharmacology and Action-Spectra of Photochromic Open-Channel Blockers

Publication: Fehrentz, T.[6]; Kuttruff, C. A.[6]; Huber, F. M. E.; Kienzler, M.; Mayer, P.; Trauner, D. *ChemBioChem* **2012**, *13*, 1746–1749.

[6] These authors contributed equally to this work.

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DOI: 10.1002/cbic.201200216

Exploring the Pharmacology and Action Spectra of Photochromic Open-Channel Blockers

Timm Fehrentz, Christian A. Kuttruff, Florian M. E. Huber, Michael A. Kienzler, Peter Mayer, and Dirk Trauner*[a]

Photochromic ligands (PCLs) act as light-dependent agonists or antagonists of receptors that allow for control of neuronal activity with high temporal and spatial precision.[1] An important subclass of PCLs is represented by photochromic ion channel blockers, which have been successfully applied on voltage-gated potassium (K_v) and sodium (Na_v) channels.[2] These channels are evolutionarily related and share similar overall architectures, despite marked differences in ion permeabilities and subunit stoichiometries.[3] Ions enter or leave a voltage-gated ion channel through selectivity filter on the extracellular side, which is connected to a water-filled inner cavity. The path from this inner cavity to the intracellular milieu is controlled by a voltage gate, which opens and closes in response to changes in the membrane potential. Local anesthetics such as lidocaine or procaine bind in the inner cavity below the selectivity filter, preventing the permeation of cations along their electrochemical gradients.[4] Because the voltage gate has to open before these molecules can reach the inner cavity, they are often referred as "use-dependent" or "open channel blockers".[4–5]

We recently introduced a family of azobenzenes that are essentially photochromic versions of lidocaine.[5b] One of our most effective compounds is a simple symmetric molecule termed QAQ, which functions as a light-dependent blocker of a wide variety of K_v and Na_v channels (Figure 1).[2c] Structurally, QAQ consists of two quaternary ammonium ions derived from glycine, linked to a 4,4′-azodianiline core through two amide bonds. Because of its double charge, QAQ is membrane-impermeant and only infiltrates pain-sensing neurons that express endogenous import channels.[2c,6] Other cells can be loaded with QAQ more invasively with a patch pipette. QAQ blocks voltage-gated Na_v and K_v channels in its *trans* form (of the azobenzene photoswitch), but not in its *cis* form. These channels underlie the initiation and propagation of action potentials (APs).[3a,4a,7] QAQ thus enables reversible optical silencing of neuronal activity and acts as a light-sensitive analgesic once applied to neural tissues. As such, it is a useful tool for studying signaling mechanisms in acute and chronic pain.[2c]

Although QAQ proved to be an effective tool for the control of neuronal activity, its photophysical properties needed to be optimized. As a diacyl derivative of azodianiline, QAQ can be most readily switched to its *cis* form with 380 nm light and back to its *trans* form with 500 nm light. The short wavelengths used to deactivate it, however, are not ideal for physiological applications, for which tissue penetration and associated phototoxicity is a primary concern. Woolley has recently introduced a class of azobenzene photoswitches that are substituted in the 2,2′- and 6,6′-positions with electron-donating substituents and show spectra red-shifted with respect to their unsubstituted analogues.[8] Trying to maintain the symmetrical nature of QAQ, we decided to modulate these substitutions in order to red-shift the action spectrum of the photochromic blocker. The question was whether these structural changes on the azobenzene core would be tolerated by the confined inner cavities of the channel proteins while allowing the use of longer-wavelength light to actuate the switch.

To address this question, we synthesized four QAQ derivatives bearing electron-donating substituents in the indicated positions: 2,2′-dimethoxy-QAQ (**1**, Figure 1), 2,2′-dimethylamino-QAQ (**2**), 2,2′-dimorpholino-QAQ (**3**) and 2,2′-*N*-dimethylpiperazine-QAQ (**4**). To allow better discrimination of steric and electronic influences, we also synthesized the methyl-substituted derivatives 2,2′-dimethyl-QAQ (**5**) and 2,2′,6,6′-tetramethyl-QAQ (**6**). The synthesis of these compounds was achieved through the use of short sequences based on diazonium coupling or oxidative coupling to generate the azobenzene core, followed by amide bond formation (see the Supporting Information).

With compounds **1**–**6** in hand, we first characterized their abilities to induce light-dependent blocking of the inactivation-removed Shaker K^+ channel (Shaker-IR),[9] a voltage-gated K^+ channel (Figure 2). Compounds were loaded into HEK293 cells expressing Shaker-IR by patch pipette. The measurements were performed after a short equilibration period of the cytosol and pipette solution.

Application of compounds **1**, **5** and **6** permitted reversible light control of Shaker-IR K^+ current (Figure 2). Derivatives **1**, **5** and **6** resulted in 31.8% ±5.7% (350 μM, $n=3$ cells), 64.7% ±5.5% (100 μM, $n=3$ cells) and 67.8% ±4.8% (100 μM, $n=5$ cells) light-induced voltage-dependent blocking of the channel current, respectively (Figures 2 and 3). The *trans* states of all active compounds induced blocking, whereas their *cis* states reversed the effect. Derivatives **5** and **6** were converted into their *cis* and *trans* states with 380 and 500 nm light, respectively.

Compound **1** is a red-shifted azobenzene PCL[8a] with an absorption maximum $\lambda_{max}=416$ nm. Irradiation with 420 nm light was used to convert the thermally stable *trans* form into the *cis* state. Thermal relaxation in the dark was used to revert compound **1** into its *trans* state. Here, the relative channel current follows the equation:

$$y_0 + A_1 \exp(-x/\tau_1) + A_2 \exp(-x/\tau_2)$$

[a] T. Fehrentz,[+] C. A. Kuttruff,[+] F. M. E. Huber, Dr. M. A. Kienzler, Dr. P. Mayer, Prof. Dr. D. Trauner
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E-mail: Dirk.Trauner@lmu.de

[+] These authors contributed equally to this work.

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/cbic.201200216.

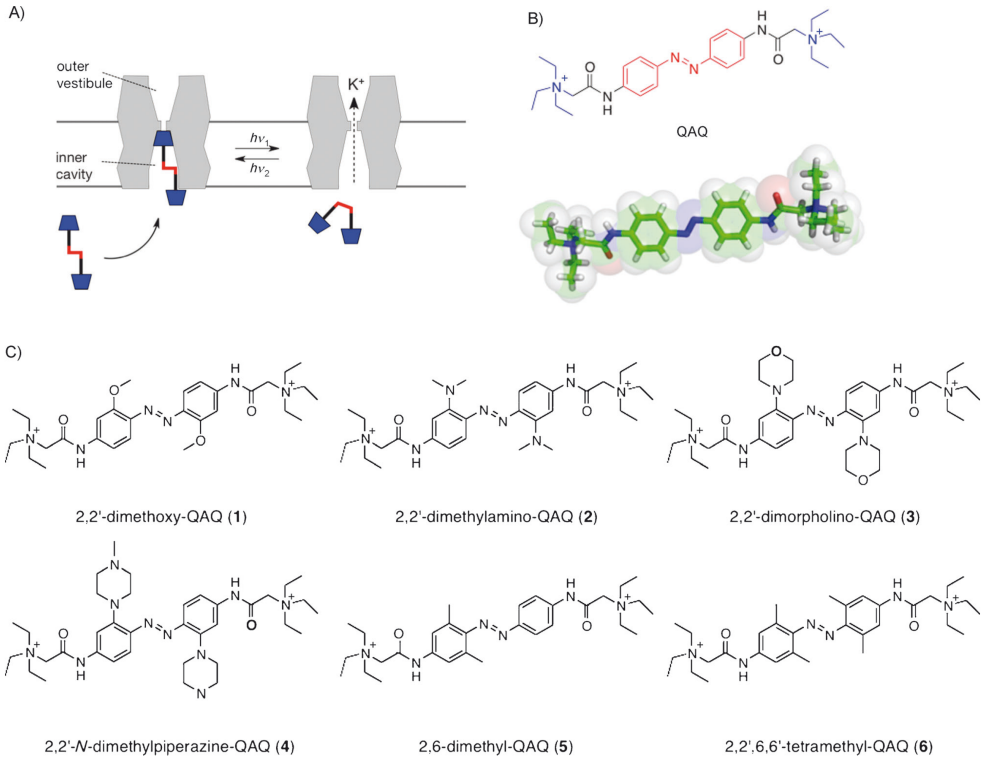

Figure 1. A) QAQ and derivatives act as light-controlled open channel blockers. Schematic depiction of light-induced *trans* blocking and *cis* unblocking of voltage-gated ion channels by QAQ derivatives. The *trans* state of the photoswitch prevents ionic current, whereas the *cis* state restores ionic conductance. B) Chemical and crystal structure of the parent molecule QAQ. The distance between the quaternary ammonium groups is approximately 18.9 Å. C) QAQ derivatives introduced in this study.

where $y_0 = 0.649 \pm 0.02$, $A_1 = 0.190 \pm 0.010$, $A_2 = 0.160 \pm 0.014$, $\tau_1 = 1.948 \pm 0.226$ s and $\tau_2 = 39.454 \pm 10.8$ s (see the Supporting Information).

In contrast, thermal relaxation of compound **1** in DMSO shows slower kinetics, the data for which were fitted to the equation:

$$y_0 + A \exp(-x/\tau),$$

where $y_0 = 0.006 \pm 0.002$, $A = 0.978 \pm 0.004$ and $\tau = 4.61 \pm 0.040$ min (see the Supporting Information). The action spectrum of compound **1** indicates that wavelengths between 350 nm–500 nm allow for *trans*-to-*cis* conversion (see the Supporting Information).

Compounds **2**, **3** and **4** showed no voltage-dependent blocking and thus no photoregulation of the Shaker-IR current.

The finding that QAQ acts on both Na_v and K_v channels[2c] prompted us to investigate whether QAQ derivatives also block Na_v channels. We tested compounds **1**, **5** and **6** on NG108-15 cells, a mouse neuroblastoma cell line that endogenously expresses Na_v channel subtypes $Na_v1.1$–1.4, 1.6 and 1.7.[10] After delivery into cells by patch pipette, all three compounds reversibly blocked voltage-induced Na^+ current in a light-dependent manner (Figure 4).

As in the case with Shaker-IR, blocking was induced by the *trans* states of all three QAQ derivatives. Compounds **1**, **5** and **6** resulted in 38.6% ±6.5% (1 mM, $n = 4$ cells), 75.5% ±1.8% (50 μM, $n = 3$ cells) and 46.3% ±4.7% (100 μM, $n = 4$ cells) photoregulation of Na^+ peak current, respectively.

Na_v and K_v channels play a crucial role in the initiation and propagation of APs in neurons.[4a,7]

Application of compounds **1, 5** and **6** into cortical pyramidal neurons of mouse slices by patch pipette allowed (after injection of a depolarizing current pulse) modulation of AP firing in a light-dependent manner (Figure 4).

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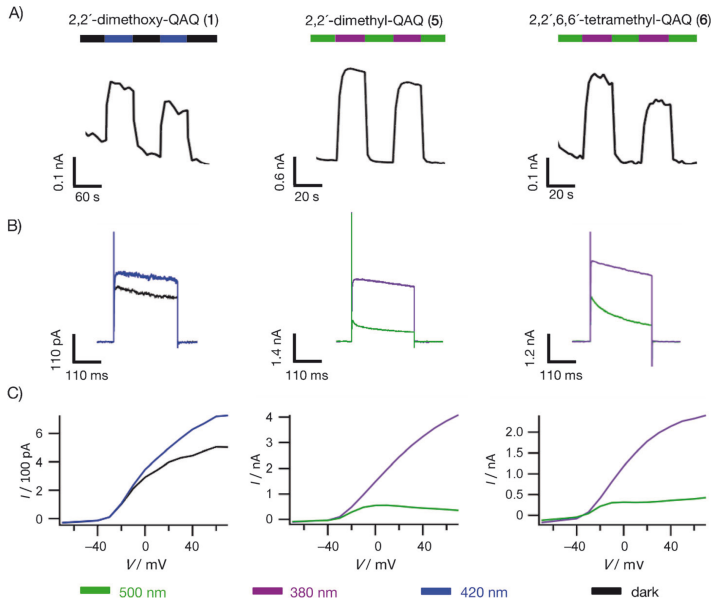

Figure 2. Application of QAQ derivatives on the internal side of a K_v channel subtype. from left to right: compounds **1** (350 μM), **5** (100 μM) and **6** (100 μM). Purple, green, blue and dark lines indicate irradiation with 380 nm light, 500 nm light and 420 nm light and darkness, respectively. A) Reversible photosensitizing of Shaker-IR K^+ current. Individual amplitudes of voltage-clamp recordings, at different irradiation wavelengths, are connected by a line. B) Comparison of single Shaker current traces under indicated wavelength, elicited by a depolarizing pulse from a holding potential of −70 mV to +40 mV for 250 ms. C) Current/voltage-dependent *trans* blocking and *cis* unblocking at indicated wavelengths from −50 mV to +70 mV.

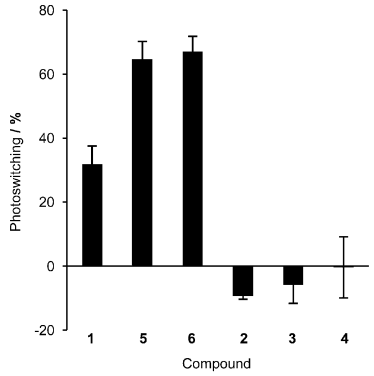

Figure 3. Quantification of QAQ derivative photoswitching on Shaker-IR. Percent photoswitching is defined as $I_{500\,nm(30\,s)}-I_{300(30\,s)}/I_{380(30\,s)}$. Derivative **1** resulted in 31.8% ±5.7% (350 μM, $n=3$ cells), derivative **5** in 64.7% ±5.5% (100 μM, $n=3$ cells) and derivative **6** in 67.8% ±4.8% (100 μM, $n=5$ cells) photoswitching, respectively, whereas derivative **2** resulted in −9.3% ±1% (500 μM, $n=3$ cells), derivative **3** in −5.8% ±5.8% (500 μM, $n=4$ cells) and derivative **4** in −0.4% ±9.8% (500 μM, $n=4$ cells) photoswitching, respectively. Negative photoswitching is due to a recording artifact resulting from current rundown.

The *trans* states of all three compounds silenced neuronal excitability, whereas the *cis* states unblocked Na_v and K_v channels, restoring AP firing. A single spike remained under *trans* recording conditions. This can be explained by the principle of open-channel block: that is, the delay between channel opening and blocker accumulation within the pore that is sufficient to trigger a single AP.[2c]

In summary, we have shown that the useful photochromic channel blocker QAQ can be further substituted at its azobenzene core. Its derivatives **1**, **5** and **6** allowed rapid reversible control of Na_v and K_v channel conductance, whereas the derivatives bearing larger substituents showed no blocking of channel current. Of the three blockers identified, only compound **1** shows a red-shifted action spectrum. Our data indicate that either the inner vestibules or the open gates leading into the inner vestibules of the Shaker-IR and Na_v channels do not tolerate QAQ derivatives bearing substituents larger than a methoxy group at the 2,2′-*ortho* positions. The higher concentration of compound **1** needed to elicit photoswitching can be interpreted as lower efficacy of the compound towards the channel, which might be a consequence of its increased size. Our study has provided three new photochromic blockers of voltage-gated channels, which might help in probing the influence of Na_v and K_v channels on dendritic signaling in neurons.[11] Moreover, they could serve as tools to unravel molecular mechanisms of pain.[2c, 12]

Experimental Section

See the Supporting Information.

Acknowledgements

We would like to thank undergraduate researcher Edris Parsa for his assistance. This work was funded by the Center of Integrated Protein Science, Munich (CIPSM) and the Deutsche Forschungsgemeinschaft (SFB 749).

Keywords: anesthetics · azo compounds · ion channels · neurochemistry · photopharmacology

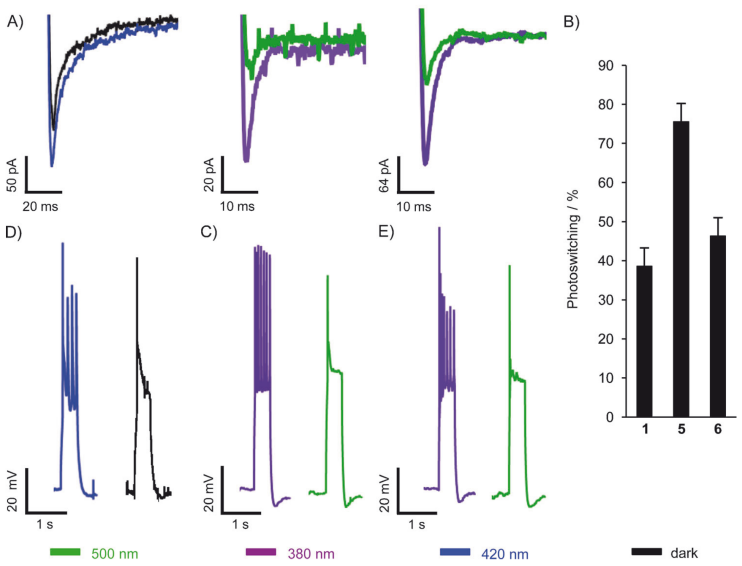

Figure 4. QAQ derivatives act as reversible internal Na_v channel blockers and control activity of cortical pyramidal neurons. Recording artifacts have been removed for clarity. A) From left to right: compound **1** (1 mM), compound **5** (50 μM) and compound **6** (100 μM) induce light-dependent Na_v current blocking of NG108-15 cells. B) Quantification of photoswitching induced by QAQ derivatives. Amount of photosensitization of Na_v current is defined as $I_{peak\_current\_380\ nm\ (10\ s)} - I_{peak\_current\_500\ nm\ (10\ s)})/I_{peak\_current\_380\ nm\ (10\ s)}$. C)–E) Reversible silencing of neuronal AP trains, evoked by current injection of approximately 150 pA (400 ms). C) Derivative **1** (1 mM). D) Derivative **5** (50 μM). E) Derivative **6** (100 μM). Resting potentials of all neurons measured were about −75 to −80 mV.

[1] a) E. Bartels, N. H. Wassermann, B. F. Erlanger, *Proc. Natl. Acad. Sci. USA* **1971**, *68*, 1820–1823; b) T. Fehrentz, M. Schönberger, D. Trauner, *Angew. Chem.* **2011**, *123*, 12362–12390; *Angew. Chem. Int. Ed.* **2011**, *50*, 12156–12182; c) M. Volgraf, P. Gorostiza, S. Szobota, M. R. Helix, E. Y. Isacoff, D. Trauner, *J. Am. Chem. Soc.* **2007**, *129*, 260–261.

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Supporting Information

Exploring the Pharmacology and Action Spectra of Photochromic Open-Channel Blockers

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Supplementary Information

Index:

General Experimental Details. Unless stated otherwise, all reactions were performed in oven-dried or flame-dried glassware under a positive pressure of nitrogen. Commercial reagents and solvents were used as received with the following exceptions. Tetrahydrofuran (THF) was distilled from benzophenone and sodium immediately prior to use. Triethylamine, diisopropylamine and diisopropylethylamine were distilled over calcium hydride immediately before use. Reactions were magnetically stirred and monitored by NMR spectroscopy or analytical thin-layer chromatography (TLC) using E. Merck 0.25 mm silica gel 60 F_{254} precoated glass plates. TLC plates were visualized by exposure to ultraviolet light (UV, 254 nm) and/or exposure to an aqueous solution of ceric ammoniummolybdate (CAM), an aqueous solution of potassium permanganate ($KMnO_4$), an acidic solution of vanillin or a solution of ninhydrin in ethanol followed by heating with a heat gun. Flash column chromatography was performed as described by Still *et al.* employing silica gel (60 Å, 40-63 μm, Merck) and a forced flow of eluant at 1.3-1.5 bar pressure.[1] Reversed phase column chromatography was carried out with Waters Prep C_{18} (55–105 μm, 125 Å) silica gel. Yields refer to chromatographically and spectroscopically (^{1}H and ^{13}C NMR) pure material.

Instrumentation. Proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded on Varian VNMRS 300, VNMRS 400, INOVA 400 or VNMRS 600 spectrometers. Proton chemical shifts are expressed in parts per million (δ scale) and are calibrated using residual undeuterated solvent as an internal reference ($CDCl_3$: δ 7.26, DMSO-d_6: δ 2.50, CD_3OD: δ 3.31). Data for ^{1}H NMR spectra are reported as follows: chemical shift (δ ppm) (multiplicity, coupling constant (Hz), integration). Multiplicities are reported as follows: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, *br* = broad, app = apparent, or combinations thereof. Carbon nuclear magnetic resonance (^{13}C NMR) spectra were recorded on Varian VNMRS 300, VNMRS 400, INOVA 400 or VNMRS 600 spectrometers. Carbon chemical shifts are expressed in parts per million (δ scale) and are referenced to the carbon resonances of the solvent ($CDCl_3$: δ 77.0, DMSO-d_6: δ 39.5, CD_3OD: δ 49.0). Infrared (IR) spectra were recorded on a Perkin Elmer Spectrum BX II (FTIR System). IR data is reported in frequency of absorption (cm^{-1}). Mass spectroscopy (MS) experiments were performed on a Thermo Finnigan MAT 95 (EI), a Thermo Finnigan LTQ FT (ESI) or a JEOL JMS-700 (FAB) instrument. UV/Visible spectra were recorded on a Varian Cary 50 Scan UV-Visible Spectrophotometer using STARNA 29/B/12 quartz cuvettes with 10 mm section thickness.

[1] Still, W. C.; Kahn, M.; Mitra, A. *J. Org. Chem.* **1978**, *43*, 2923–2925.

Electrophysiology and cell culture. HEK293 and NG108-15 cells haven been cultured and cortical slice preparation was performed as described before.[2,3]

Patch clamp recordings and analysis have been performed with a standard electrophysiological setup, including an HEKA Patch Clamp EPC10 USB amplifier and patch master software. Measurements were recorded in whole cell mode. Pipette (Science Products GB200-F-8P with filament) resistance varied between 4-6 MΩ. Irradiation of samples have been performed with a TILL Photonics Polychrome 5000 monochromator, through a Nicon Fluor 60x/1.00w objective.[2]

Extracellular potassium current recording solution contained in mM: 138 NaCl, 1.5 KCl, 1.2 $MgCl_2$, 2.5 $CaCl_2$, 5 HEPES free acid, 10 glucose and pH was adjusted to 7.4. Extracellular sodium current recording solution contained in mM: 145 NaCl, 0.5 $CdCl_2$, 2 $CaCl_2$, 5 HEPES free acid, 5 glucose and pH was adjusted to 7.4. Internal potassium current recording solution contained in mM: 10 NaCl, 135 K gluconate, 10 HEPES free acid, 2 $MgCl_2$, 2 MgATP, 1 EGTA and pH was adjusted to 7.4. Internal sodium current recording solution contained in mM: 30 NaCl, 100 CsCl, 10 HEPES free acid, 2 $MgCl_2$, 1 $CaCl_2$, 2 MgATP 0.05 GTP, 10 EGTA, 5 glucose and pH was adjusted (CsOH) to 7.3. Ringer solution contained in mM: 96 NaCl, 2 KCl, 1 $MgCl_2$, 1.8 $CaCl_2$, 5 HEPES free acid and pH was adjusted to 7.4.

Photoswitches were dissolved in internal solution to give the final working concentrations. DMSO concentration in internal solutions was below 0.1%. After patching cells, internal solution and cytosol were allowed to equilibrate for about 2–3 min. A typical voltage clamp protocol to record Shaker-IR current depolarized, in a looped manner, the membrane from its holding potential of -70 mV to +40 mV for 250 ms. The protocol was applied with a frequency of 0.5 Hz and the depolarization was initiated 250 ms after protocol started. Recordings were accompanied by illumination at a defined wavelength, which was changed every 30 cycles. To record current-voltage (IV) curves of Shaker-IR, a protocol hold the cell at -70 mV and depolarized the cell in a loop sequence to values between -50 mV and +70 mV, in 10 mV intervals. Illumination wavelengths were changed for each IV protocol.

The voltage clamp protocol to record Na_v channel current, hold the cell at -100 mV and depolarized the cell, for 250 ms, to -10 mV. The depolarisation was initiated 250 ms after the

[2] Mourot, A.; Fehrentz, T.; Le Feuvre, Y.; Smith, C. M.; Herold, C.; Dalkara, F.; Nagy, F.; Trauner, D.; Kramer, R. H. *Nat. Methods* **2012**, Article *ASAP*.

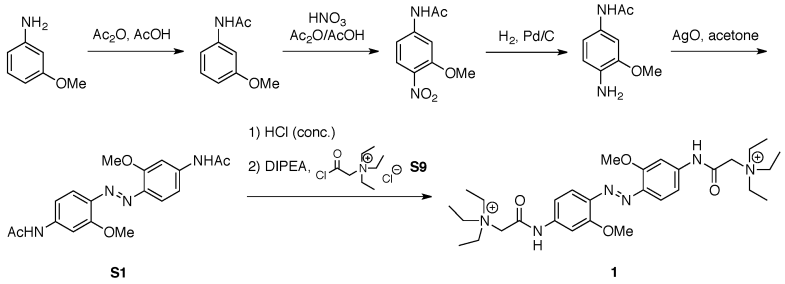
[3] Bischofberger, J.; Engel, D.; Li, L.; Geiger, J. R. P.; Jonas, P. *Nat. Prot.* **2006**, *1*, 2075–2081.

beginning of the protocol, which was applied at a frequency of 0.5 Hz. Recordings were accompanied by changing irradiation wavelength every 10 cycles. Recording of APs was performed in current clamp mode. Single current pulses with the same strength were injected into cortical pyramidal neurons to elicit robust AP firing in the presence of QAQ derivatives in the *cis* state but not in the *trans* state. All data presented have been analyzed as averages ± s.e.m.

Syntheses.

Synthesis of 2,2'-Dimethoxy-QAQ (1)

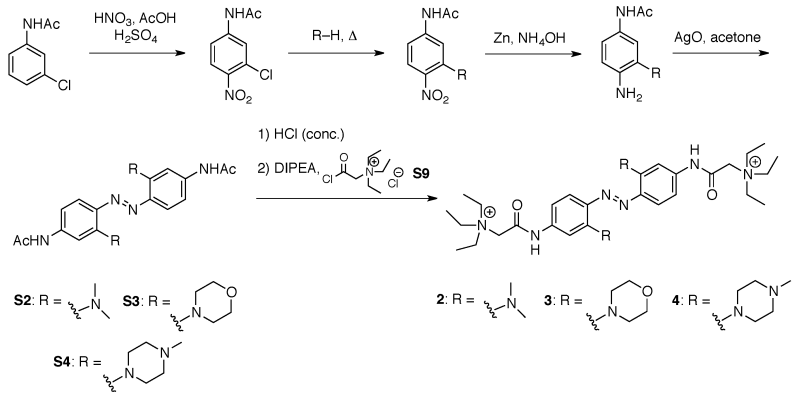
1 was synthesized as outlined in the Scheme below. The synthesis of its precursor **S1** has been described previously.[4]



Synthesis of 2,2'-Dimethylamino-QAQ (2), 2,2'-Dimorpholino-QAQ (3) and 2,2'-Dimethylpiperazine-QAQ (4)

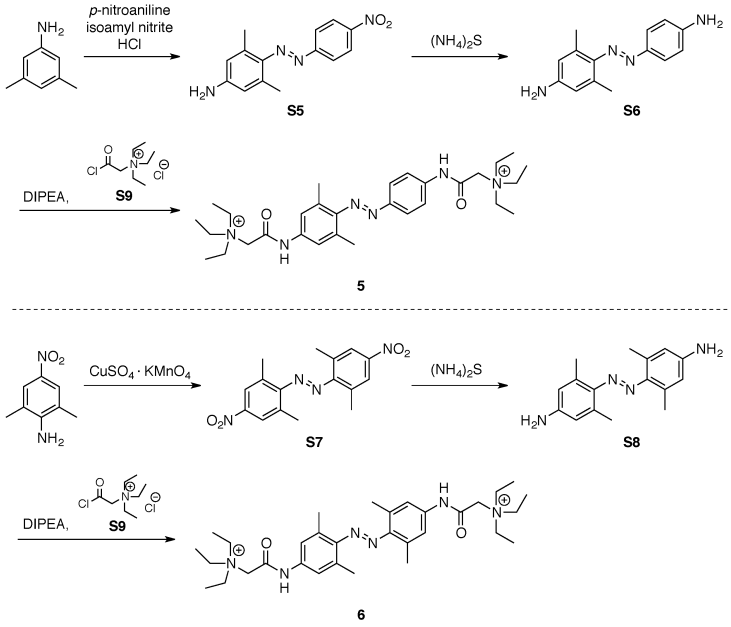
Compounds **2**, **3** and **4** were synthesized from the corresponding bisacetamides **S2**, **S3** and **S4**, whose syntheses were described previously.[5]

[4] Beharry, A. A.; Sadovski, O.; Woolley, G. A. *J. Am. Chem. Soc.* **2011**, *133*, 19684–19687.
[5] Sadovski, O.; Beharry, A. A.; Zhang, F.; Woolley, G. A. *Angew. Chem. Int. Ed.* **2009**, *48*, 1484–1486.

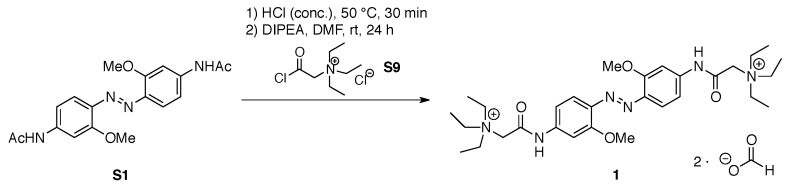


Synthesis of 2,6-Dimethyl-QAQ (5) and 2,2',6,6'-Tetramethyl-QAQ (6)

Compounds **5** and **6** were synthesized as outlined in the Scheme below.



Synthetic procedures.



2,2'-Dimethoxy-QAQ (1)

Diazene **S1** (14 mg, 39 µmol, 1.0 equiv.) was dissolved in conc. HCl (2.5 mL) and the purple reaction mixture was heated to 50 °C for 30 min. The reaction mixture was cooled to rt and the solvent removed under high vacuum. The residue was dissolved in dry DMF (2 mL) and DIPEA (66 µL, 0.39 mmol, 10.0 equiv.) was added dropwise. The resulting clear orange solution was then added to a solution of **S9**[6] (84 mg, 0.39 mmol, 10.0 equiv.) in dry DMF (2 mL) at 0 °C. The reaction mixture was allowed to warm to rt, stirred overnight and MeOH (1 mL) was added. All volatiles were subsequently removed under high vacuum. The crude product was purified by HPLC (Varian Dynamax 250×21.4 mm Microsorb 60-8 C_{18} column equipped with a Dynamax HPLC guard column operating on a Varian PrepStar HPLC system; H_2O/MeCN/0.1% formic acid; 20 mL/min; gradient program: t = 0 min, 5% MeCN; t = 30 min, 95% MeCN, t_R(**1**) = 12.05 min.) to yield dimethyl-QAQ formate salt **1** (18 mg, 2.78 µmol, 71%) as a red solid.

^{1}H NMR (400 MHz, CD_3OD) δ: 8.58 (*br* s, 2 H), 7.72 (d, *J* = 1.8 Hz, 2 H), 7.65 (d, *J* = 8.8 Hz, 2 H), 7.15 (dd, *J* = 8.8, 1.1 Hz, 2 H), 4.22 (s, 4 H), 4.02 (s, 6 H), 3.69 (q, *J* = 7.2 Hz, 12 H), 1.40 (t, *J* = 7.2 Hz, 18 H).

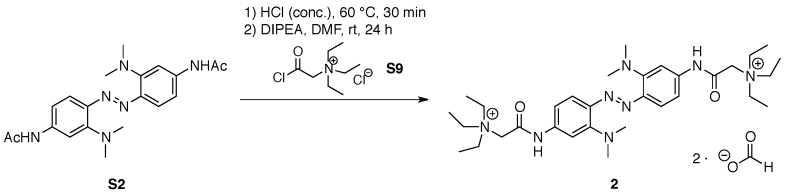
^{13}C NMR (100 MHz, CD_3OD) δ: 163.3, 158.9, 142.8, 140.7, 118.7, 113.1, 105.4, 57.6, 56.8, 55.8, 8.0.

IR (Diamond-ATR, neat) $\tilde{\nu}$: 3383, 2988, 2941, 2779, 2684, 1688, 1626, 1583, 1453, 1412, 1372, 1342, 1272, 1249, 1201, 1170, 1127, 1027, 1127, 1086, 1027, 1010, 855, 757 cm^{-1}.

HRMS (EI) calcd for $C_{30}H_{48}O_4N_6$ $[M]^{+\bullet}$: 556.3726; found: 556.3723.

UV-Vis (DPBS, pH = 7.4): λ_{max} = 394 nm.

[6] Fortin, D. L.; Banghart, M. R.; Dunn, T. W.; Borges, K.; Wagenaar, D. A.; Gaudry, Q.; Karakossian, M. H.; Otis, T. S.; Kristan, W. B.; Trauner, D.; Kramer R. H. *Nature Methods* **2008**, *5*, 331–338.



2,2'-Dimethylamino-QAQ (2):

Diazene **S2** (32 mg, 0.8 µmol, 1.0 equiv.) was dissolved in conc. HCl (1.0 mL) and the dark red reaction mixture was heated to 60 °C for 30 min. The reaction mixture was cooled to rt and the solvent removed under high vacuum. The residue was dissolved in dry DMF (1.0 mL) and DIPEA (0.3 mL, 8.0 mmol, 10.0 equiv.) was added dropwise. The solution was cooled to 0 °C and a solution of **S9** (130 mg, 8.0 µmol, 10 equiv.) in dry DMF (1.0 mL) was added dropwise. The reaction mixture was allowed to warm to rt, stirred overnight and DMF was subsequently removed under high vacuum. The crude product was purified by reversed phase column chromatography (gradient: MeOH/H_2O/HCOOH = 0:1:1·10^{-3} → 2:8:8·10^{-3}) to afford dimethylamino-QAQ formate salt **2** (12 mg, 17.8 µmol, 21%) as a dark red solid.

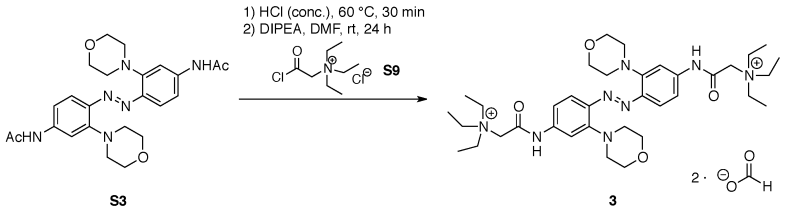
^{1}H NMR (600 MHz, CD_3OD) δ: 8.41 (*br* s, 2 H), 7.65 (d, *J* = 8.8 Hz, 2 H), 7.54 (d, *J* = 1.9 Hz, 2 H), 7.12 (dd, *J* = 8.8, 1.9 Hz, 2 H), 4.19 (s, 4 H), 3.68 (q, *J* = 7.2 Hz, 12 H), 3.06 (s, 12 H), 1.40 (t, *J* = 7.2 Hz, 18 H).

^{13}C NMR (150 MHz, CD_3OD) δ: 163.1, 152.8, 142.5, 141.6, 118.9, 113.6, 110.2, 103.3, 57.6, 55.8, 45.5, 8.0.

IR (Diamond-ATR, neat) $\tilde{\nu}$: 3255, 2987, 2948, 2780, 2683, 1685, 1659, 1579, 1477, 1456, 1404, 1371, 1342, 1307, 1250, 1200, 1173, 1157, 1115, 1051, 1007, 950, 857, 790, 774, 765, 718 cm^{-1}.

HRMS (FAB) calcd for $C_{32}H_{54}O_2N_8$ $[M]^{+\bullet}$: 582.4359; found: 582.4368.

UV-Vis (DPBS, pH = 7.4): λ_{max} = 430 nm.



2,2'-Dimorpholino-QAQ (3):

Diazene **S3** (117 mg, 0.25 mmol, 1.0 equiv.) was dissolved in conc. HCl (10 mL) and the dark red reaction mixture was heated to 60 °C for 30 min. The reaction mixture was cooled to rt and the solvent removed under high vacuum. The residue was dissolved in dry DMF (10 mL) and DIPEA (0.44 mL, 2.5 mmol, 10.0 equiv.) was added dropwise. The solution was cooled to 0 °C and a solution of **S9** (535 mg, 2.5 mmol, 10 equiv.) in dry DMF (20 mL) was added dropwise. The reaction mixture was allowed to warm to rt, stirred overnight and DMF was subsequently removed under high vacuum. The crude product was purified by reversed phase column chromatography (gradient: MeOH/H_2O/HCOOH = $1:9:9\cdot10^{-3}$ → $1.5:1:1\cdot10^{-3}$) to afford dimorpholino-QAQ formate salt **3** (52 mg, 68.7 µmol, 27%) as a dark red solid.

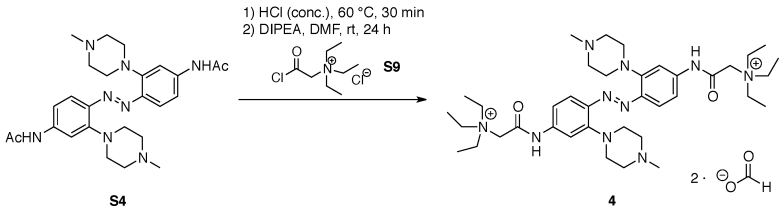
^{1}H NMR (400 MHz, CD_3OD) δ: 8.50 (*br* s, 2 H), 7.61–7.59 (m, 4 H), 7.25 (d, *J* = 8.4 Hz, 2 H), 4.21 (*br* s, 4 H), 3.92–3.90 (m, 8 H), 3.69 (q, *J* = 7.1 Hz, 12 H), 3.28–3.26 (m, 8 H), 1.40 (t, *J* = 7.2 Hz, 18 H).

^{13}C NMR (100 MHz, CD_3OD) δ: 163.3, 152.6, 143.1, 142.3, 118.5, 114.7, 111.0, 68.1, 57.6, 55.8, 54.5, 8.0.

IR (Diamond-ATR, neat) $\tilde{\nu}$: 3252, 2954, 2817, 2683, 1688, 1579, 1550, 1482, 1418, 1372, 1341, 1305, 1244, 1229, 1188, 1112, 1066, 1050, 1010, 948, 884, 811, 788, 753 cm^{-1}.

HRMS (ESI) calcd for $C_{36}H_{58}O_4N_8$ $[M]^{2+}$: 333.2285; found: 333.2287.

UV-Vis (DPBS, pH = 7.4): λ_{max} = 416 nm.



2,2'-Di-methylpiperazin-QAQ (4):

Diazene **S4** (70 mg, 0.14 mmol, 1.0 equiv.) was dissolved in conc. HCl (8 mL) and the dark red reaction mixture was heated to 60 °C for 30 min. The reaction mixture was cooled to rt and the solvent removed under high vacuum. The residue was dissolved in dry DMF (5 mL) and DIPEA (0.25 mL, 1.42 mmol, 10.0 equiv.) was added dropwise. The solution was cooled to 0 °C and a solution of **S9** (535 mg, 2.50 mmol, 10 equiv.) in dry DMF (10 mL) was added dropwise. The reaction mixture was allowed to warm to rt, stirred overnight and the solvent was subsequently removed under high vacuum. The crude product was purified by reversed

phase column chromatography (gradient: MeOH/H_2O/HCOOH = 1:10:10 · 10^{-3} → 1:2:2 · 10^{-3}) to afford di-methylpiperazin-QAQ formate salt **4** (48 mg, 61.3 µmol, 44%) as a dark red solid.

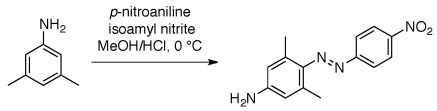
^{1}H NMR (400 MHz, CD_3OD) δ: 8.52 (*br* s, 2 H), 7.63 (d, *J* = 8.8 Hz, 2 H), 7.59 (d, *J* = 1.9 Hz, 2 H), 7.26 (dd, *J* = 8.9, 1.8 Hz, 2 H), 4.20 (s, 4 H), 3.69 (q, *J* = 7.3 Hz, 12 H), 3.38 (app s, 8 H), 2.89 (app s, 8 H), 2.53 (s, 6 H), 1.40 (t, *J* = 7.2 Hz, 18 H).

^{13}C NMR (100 MHz, CD_3OD) δ: 174.6, 163.3, 152.2, 143.1, 142.3, 118.6, 114.9, 111.4, 57.6, 55.8, 52.9, 45.5, 8.0.

IR (Diamond-ATR, neat) $\tilde{\nu}$: 3438, 2924, 2456, 1688, 1580, 1543, 1456, 1424, 1395, 1344, 1318, 1280, 1246, 1201, 1122, 1088, 1029, 981, 920, 867, 829, 789 cm^{-1}.

HRMS (ESI) calcd for $C_{38}H_{64}N_{10}O_2$ $[M]^{2+}$: 346.2602; found: 346.2604.

UV-Vis (DPBS, pH = 7.4): λ_{max} = 415 nm.



S5

4-Amino-2,6-dimethyl-4'-nitroazobenzene (S5):

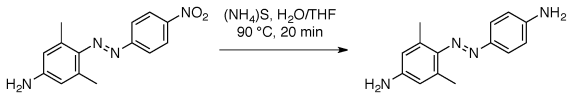
To a solution of *p*-nitroaniline (1.1 g, 7.97 mmol, 1.0 equiv.) in methanol (70 mL), conc. HCl (7 mL) was added at 0 °C and the resulting solution was stirred for 5 min under argon atmosphere. Isoamyl nitrite (1.2 mL, 8.77 mmol, 1.1 equiv.) was added and stirring was continued at 0 °C for 1 h. In a separate flask, 3,5-dimethyl aniline (966 mg, 7.97 mmol, 1.0 equiv.) was dissolved in MeOH (70 mL), cooled to 0 °C, and conc. HCl (7 mL) was added. To this mixture, the freshly prepared solution of diazonium salt was added dropwise at 0 °C over a period of 10 min. The resulting violet reaction mixture was stirred at 0 °C for 90 min, poured into aqueous $NaHCO_3$ (400 mL), and extracted with EtOAc (3 × 100 mL). The combined organic layers were dried over Na_2SO_4 and concentrated *in vacuo*. Purification by flash column chromatography (silica gel, gradient: hexanes/EtOAc = 4:1 → 3:7) yielded diazene **S5** (1.4 g, 5.18 mmol, 65%) as an orange solid.

^{1}H NMR (300 MHz, $CDCl_3$) *δ*: 8.31 (d, *J* = 9.0 Hz, 2 H), 7.85 (d, *J* = 9.0 Hz, 2 H), 6.41 (s, 2 H), 4.06 (s, 2 H), 2.54 (s, 6 H).

^{13}C NMR (100 MHz, $CDCl_3$) *δ*: 157.3, 149.5, 147.6, 142.3, 138.6, 124.9, 122.6, 115.5, 21.7.

IR (Diamond-ATR, neat) $\tilde{\nu}$: 3421, 3333, 1604, 1586, 1510, 1313, 1168, 1142, 1103 cm^{-1}.

HRMS (FAB) calcd. for $C_{14}H_{15}N_4O_2$ $[M+H]^+$: 271.1190, found: 271.1199.



S5 **S6**

2,6-Dimethyl-4,4'-diaminoazobenzene (S6):

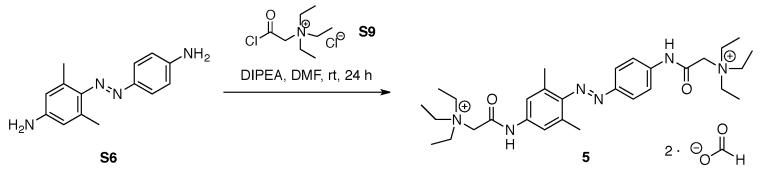
To a mixture of 4-amino-2,6-dimethyl-4'-nitroazobenzene (**S5**) (200 mg, 0.73 mmol, 1.0 equiv.) and Na_2CO_3 (345 mg, 3.26 mmol, 4.5 equiv.) in THF/H_2O (10 mL, 1:1) was added an aqueous solution of $(NH_4)_2S$ (20% w/w, 2.19 mmol, 3.0 equiv.). The reaction was heated to 90 °C for 20 min and its progress was monitored *via* TLC and if necessary additional aqueous solution of $(NH_4)_2S$ (20% w/w) was added in 0.5 equiv. portions and refluxed for further 20 min until the reaction was complete. After cooling to room temperature, the reaction was carefully acidified to pH = 7 and extracted with EtOAc (3 × 20 mL). The combined organic layers were washed with H_2O (20 mL), sat. aqueous $NaHCO_3$ (20 mL), and brine (20 mL) followed by drying over Na_2SO_4, filtration, and removal of the solvent *in vacuo*. Purification by flash column chromatography (silica gel, gradient: hexanes/EtOAc = 4:1 → 3:7) afforded diamino-azobenzene **S6** (148 mg, 0.62 mmol, 84%) as an orange solid.

^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.70 (d, J = 8.7 Hz, 2 H), 6.71 (d, J = 8.7 Hz, 2 H), 6.39 (s, 2 H), 3,82 (m, 4 H), 2.39 (s, 6 H).

^{13}C NMR (100 MHz, $CDCl_3$) δ: 148.6, 146.5, 146.5, 143.4, 134.6, 124.2, 115.6, 114.9, 20.3.

IR (Diamond-ATR, neat) $\tilde{\nu}$: 3355, 1597, 1504, 1328, 1297, 1159 cm^{-1}.

HRMS (FAB) calcd. for $C_{14}H_{16}N_4$ $[M]^{+\bullet}$: 240.1375, found: 240.1371.



S6 **5**

2,6-Dimethyl-QAQ (5):

Acyl chloride **S9** (428 mg, 2.0 mmol, 6.7 equiv.) was dissolved in dry DMF (10 mL) and added dropwise to a solution of diaminoazobenzene **S6** (74 mg, 0.3 mmol, 1.0 equiv.) in dry DMF (10 mL) and DIPEA (0.8 mL, 4.6 mmol, 15.3 equiv.) over the course of 30 min at 0 °C.

The reaction mixture was allowed to warm to rt, stirred overnight and DMF was subsequently removed under high vacuum. The crude product was purified by reversed phase column chromatography (gradient: MeOH/H_2O/HCOOH = 1:41:41·10^{-3} → 3:20:2·10^{-2}) to yield dimethyl-QAQ formate salt **5** (72 mg, 117 µmol, 39%) as a red solid.

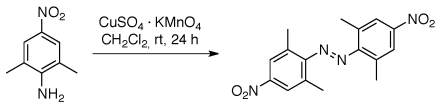
^{1}H NMR (400 MHz, CD_3OD) δ: 8.68 (*br* s, 2 H), 7.88–7.81 (m, 4 H), 7.45 (s, 2 H), 4.27 (s, 2 H), 4.23 (s, 2 H), 3.70–3.63 (m, 12 H), 2.39 (s, 6 H), 1.40–1.37 (m, 18 H).

^{13}C NMR (100 MHz, CD_3OD) δ: 163.5, 163.3, 151.1, 148.8, 141.7, 139.1, 134.3, 124.4, 121.8, 121.7, 57.8, 57.7, 55.9, 20.0, 8.2, 8.1.

IR (Diamond-ATR, neat) $\tilde{\nu}$: 3364, 2989, 1688, 1581, 1556, 1476, 1343, 1320, 1153 cm^{-1}.

HRMS (ESI) calcd. for $C_{30}H_{47}N_6O_2$ $[M-H]^+$: 523.3755, found: 523.3752.

UV-Vis (DPBS, pH = 7.4): λ_{max} = 340 nm.



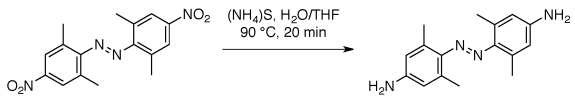
S7

2,2',6,6'-Tetramethyl-4,4'-dinitroazobenzene (S7):

To a solution of 2,6-dimethyl-4-nitroaniline (11.23 g, 68 mmol, 1.0 equiv.) in CH_2Cl_2 (700 mL) was added a pre-ground mixture of $CuSO_4 \cdot 5H_2O$ (65.64 g, 263 mmol, 3.9 equiv.) and $KMnO_4$ (62.32 g, 394 mmol, 5.8 equiv.) with strong stirring. The brown suspension was allowed to stir for 24 h and was then filtered through a mixture of celite and silica gel while rinsing with CH_2Cl_2 and concentrated *in vacuo*. Subsequent purification by flash column chromatography (silica gel, gradient: CH_2Cl_2/hexanes = 3:20 → 1:1) provided diazene **S7** (5.54 g, 17 mmol, 25%) as a dark pink solid.

^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.06 (s, 4 H), 2.47 (s, 12 H).

HRMS (EI) calcd. for $C_{16}H_{16}N_4O_4$ $[M]^{+\bullet}$: 328.1172, found: 328.1175.



S7 **S8**

2,2',6,6'-Tetramethyl-4,4'-diaminoazobenzene (S8):

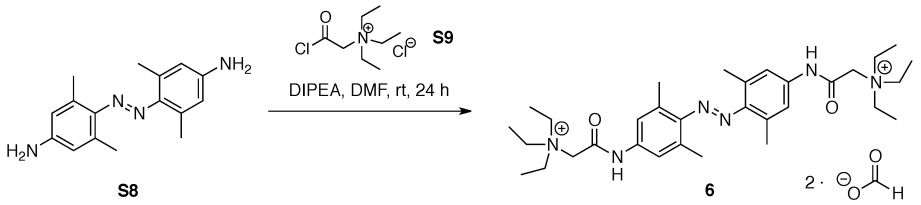
To a mixture of 2,2',6,6'-tetramethyl-4,4'-dinitroazobenzene (**S7**) (1.45 g, 4.42 mmol, 1.0 equiv.) and Na_2CO_3 (2.11 g, 19.90 mmol, 4.5 equiv.) in THF/H_2O (50 mL, 1:1) was added an aqueous solution of $(NH_4)_2S$ (20% w/w, 13.26 mmol, 3.0 equiv.). The reaction was heated to 90 °C for 20 min. The reaction progress was monitored *via* TLC and if necessary additional aqueous solution of $(NH_4)_2S$ (20% w/w) was added in 0.5 equiv. portions and refluxed for further 20 min until the reaction was complete. After cooling to room temperature, the reaction was carefully acidified to pH = 7 and extracted with EtOAc (3 × 50 mL). The combined organic layers were washed with H_2O (50 mL), sat. aqueous $NaHCO_3$ (50 mL), and brine (50 mL) followed by drying over Na_2SO_4, filtration, and removal of the solvent *in vacuo*. Purification by flash column chromatography (silica gel, EtOAc/CH_2Cl_2 = 1:99) afforded diaminoazobenzene **S8** (963 mg, 3.59 mmol, 81%) as an orange solid.

^{1}H NMR (400 MHz, $CDCl_3$) δ: 6.44 (s, 4 H), 3.76 (s, 4 H), 2.45 (s, 12 H).

^{13}C NMR (125 MHz, $CDCl_3$) δ: 146.2, 143.7, 134.7, 115.4, 21.2.

IR (Diamond-ATR, neat) $\tilde{\nu}$: 3459, 3395, 1598, 1469, 1324, 1166 cm^{-1}.

HRMS (FAB) calcd. for $C_{16}H_{20}N_4$ $[M]^{+\bullet}$: 268.1688, found: 268.1682.



2,2',6,6'-Tetramethyl-QAQ (6):

Acyl chloride **S9** (257 mg, 1.2 mmol, 1.7 equiv.) was dissolved in dry DMF (10 mL) and added dropwise to a solution of diaminoazobenzene **S8** (188 mg, 0.7 mmol, 1.0 equiv.) in dry DMF (10 mL) and DIPEA (0.24 mL, 1.4 mmol, 2.0 equiv.) over the course of 30 min at 0 °C. The reaction mixture was allowed to warm to rt, stirred overnight and DMF was subsequently removed under high vacuum. Purification of the crude product by reversed phase column chromatography (gradient: MeOH/H_2O/HCOOH = 0:1:1 · 10^{-3} → 17:20:2 · 10^{-2}) to yielded tetramethyl-QAQ formate salt **6** (168 mg, 0.3 mmol, 37%) as a red solid.

^{1}H NMR (400 MHz, CD_3OD) δ: 8.40 (*br* s, 2 H), 7.47 (s, 4 H), 4.23 (s, 4 H), 3.68 (q, J = 7.2 Hz, 12 H), 2.44 (s, 12 H), 1.40 (t, J = 7.2 Hz, 18 H).

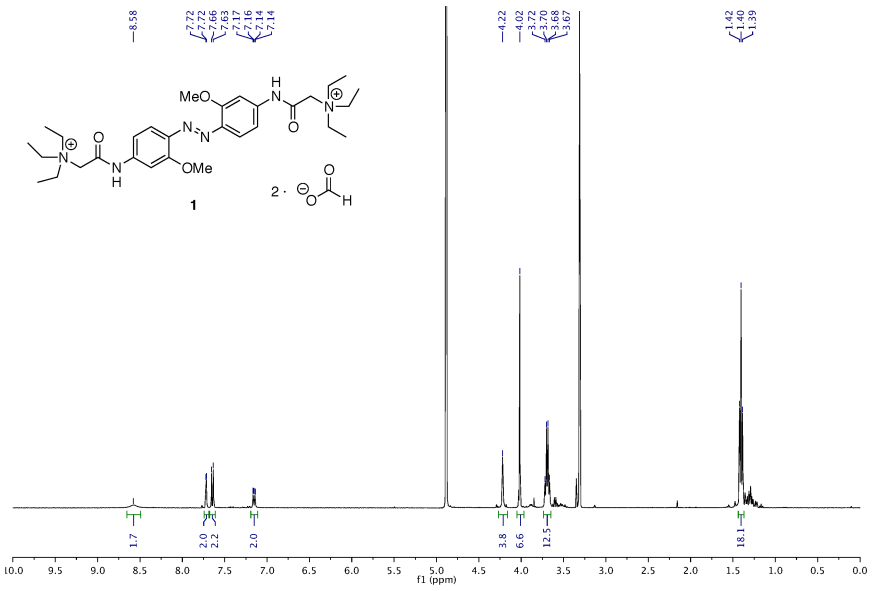
^{13}C NMR (100 MHz, CD_3OD) δ: 163.2, 149.1, 138.9, 134.3, 121.7, 55.8, 20.8, 8.0.

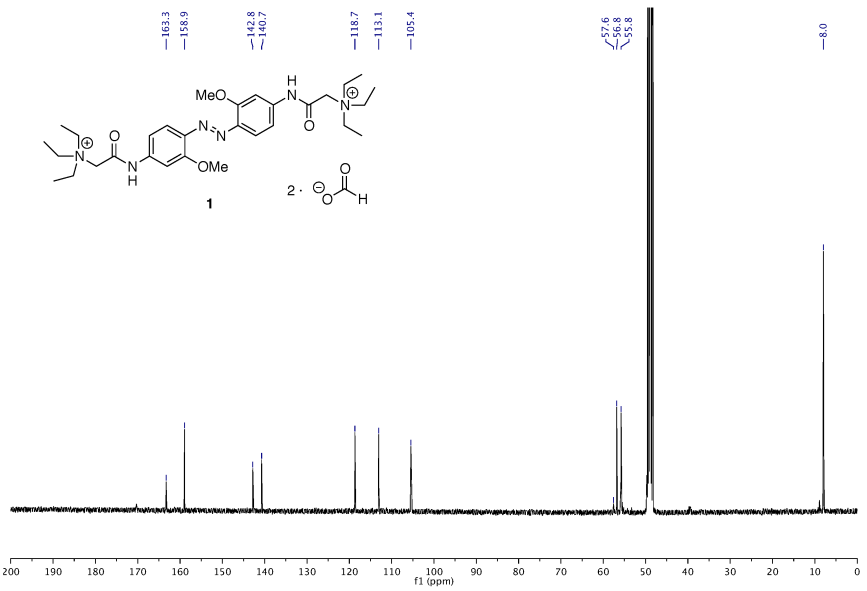
IR (Diamond-ATR, neat) $\tilde{\nu}$: 3372, 2989, 1688, 1593, 1472, 1346, 1320, 1234 cm^{-1}.

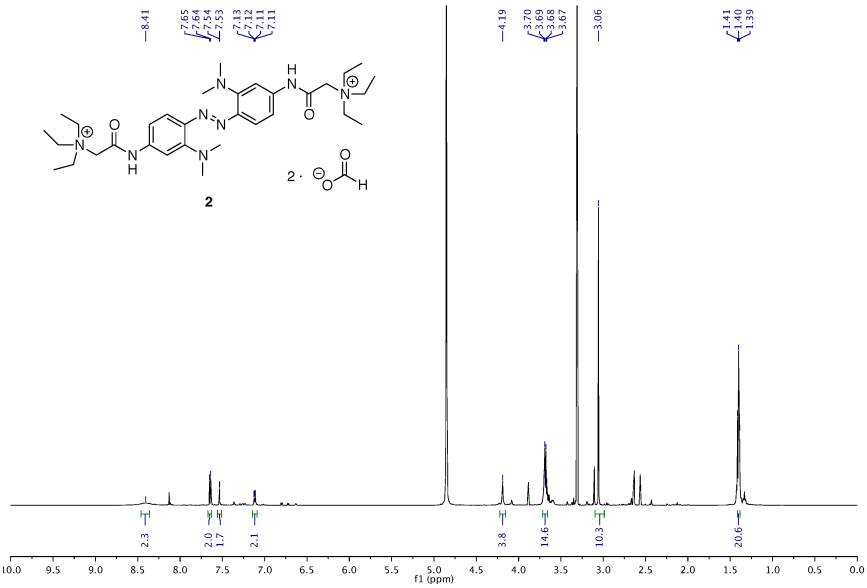
HRMS (ESI) calcd. for $C_{32}H_{51}N_6O_2$ $[M-H]^+$: 551.4068, found: 551.4071.

UV-Vis (DPBS, pH = 7.4): λ_{max} = 333 nm.

NMR spectra.




163.3
158.9
142.8
140.7
118.7
113.1
105.4
57.6
56.8
55.8
8.0
MeO
OMe
1
2 ·
f1 (ppm)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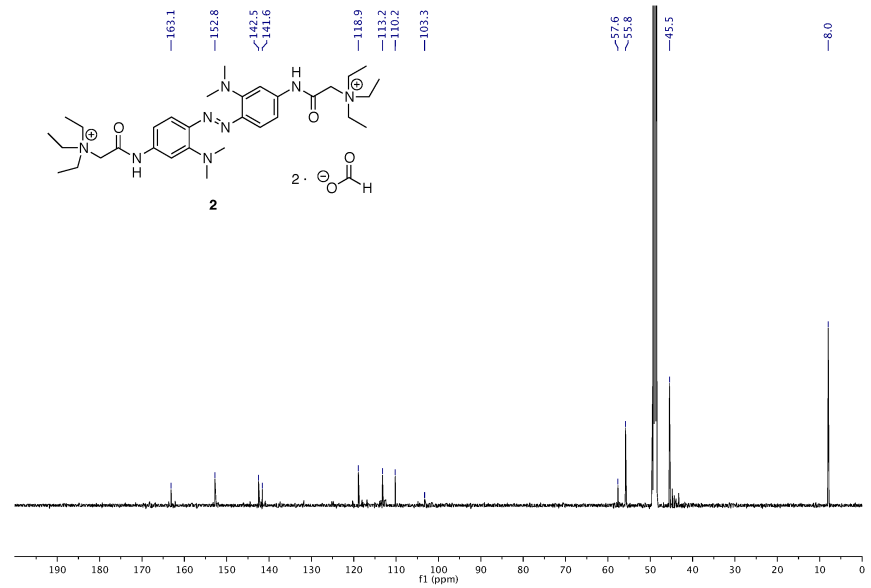
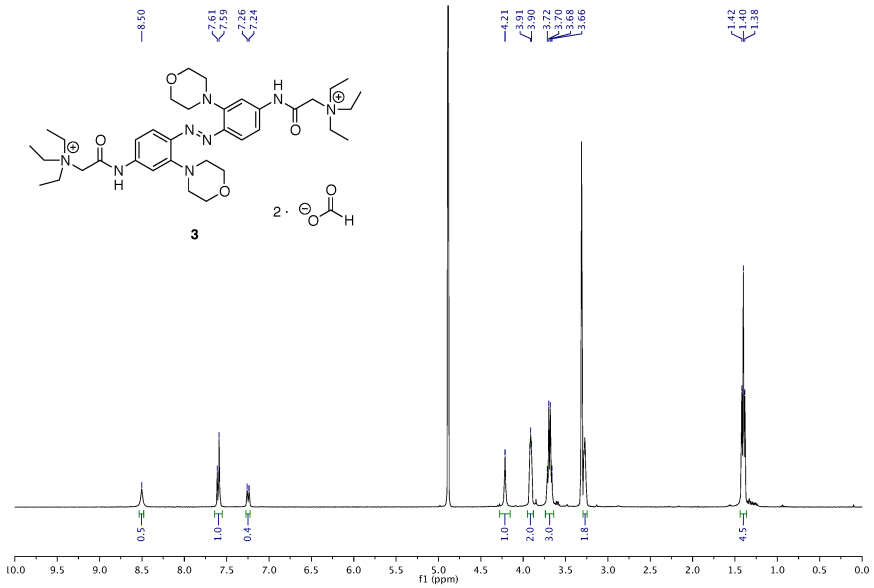
7.64
7.54
7.53
7.13
7.12
7.11
7.11
4.19
3.70
3.69
3.68
3.67
3.06
1.41
1.40
1.39
2
2 ·
2.3
2.0
1.7
2.1
3.8
14.6
10.3
20.6
f1 (ppm)


163.1
152.8
142.5
141.6
118.9
113.2
110.2
103.3
57.6
55.8
45.5
8.0
2
f1 (ppm)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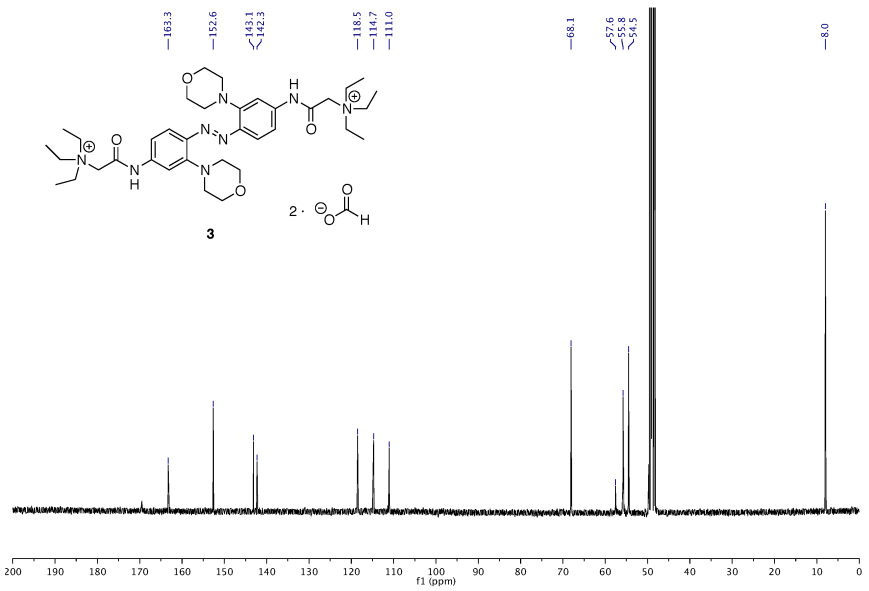
7.26
7.24
4.21
3.91
3.90
3.72
3.70
3.68
3.66
1.42
1.40
1.38
0.5
1.0
0.4
1.0
2.0
3.0
1.8
4.5
3
f1 (ppm)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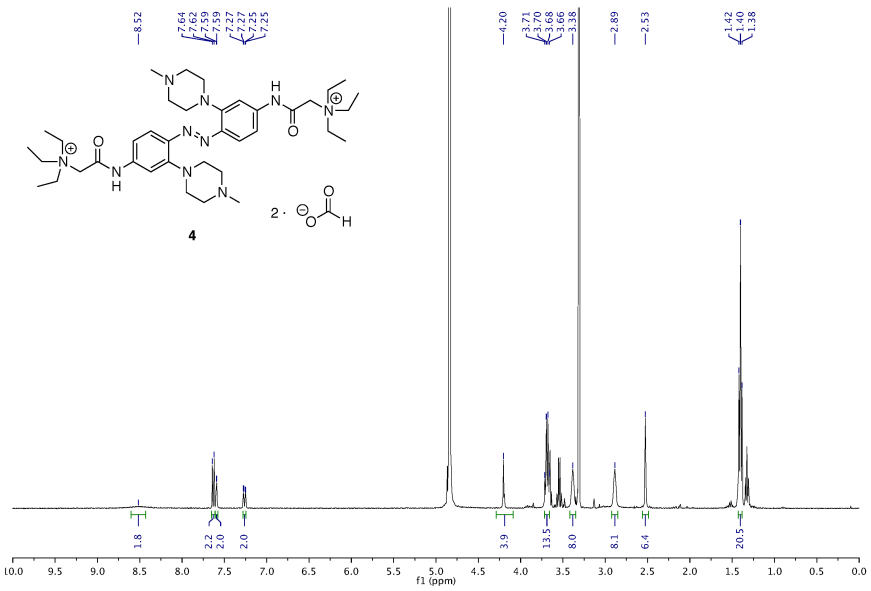
152.6
143.1
142.3
118.5
114.7
111.0
68.1
57.6
55.8
54.5
8.0
3
f1 (ppm)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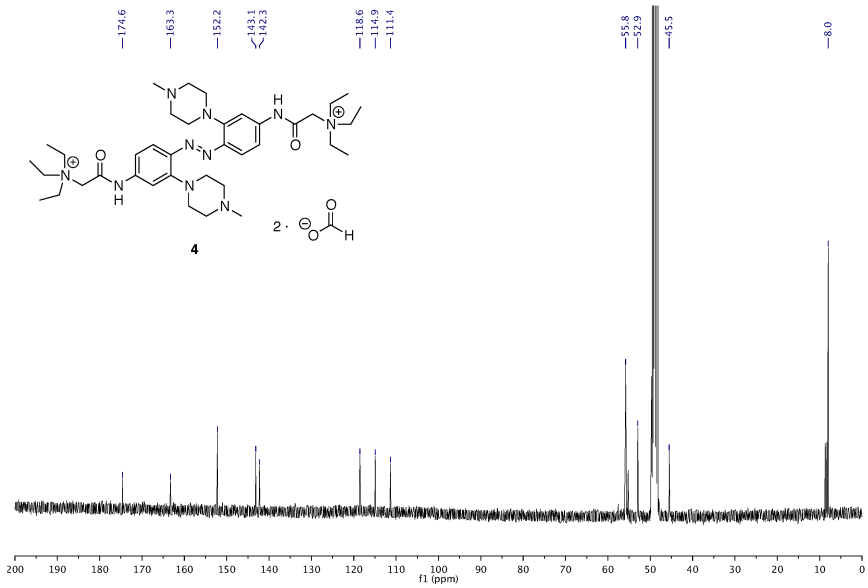
7.62
7.59
7.59
7.27
7.27
7.25
7.25
4.20
3.71
3.70
3.68
3.66
3.38
2.89
2.53
1.42
1.40
1.38
1.8
2.2
2.0
2.0
3.9
13.5
8.0
8.1
6.4
20.5
4
f1 (ppm)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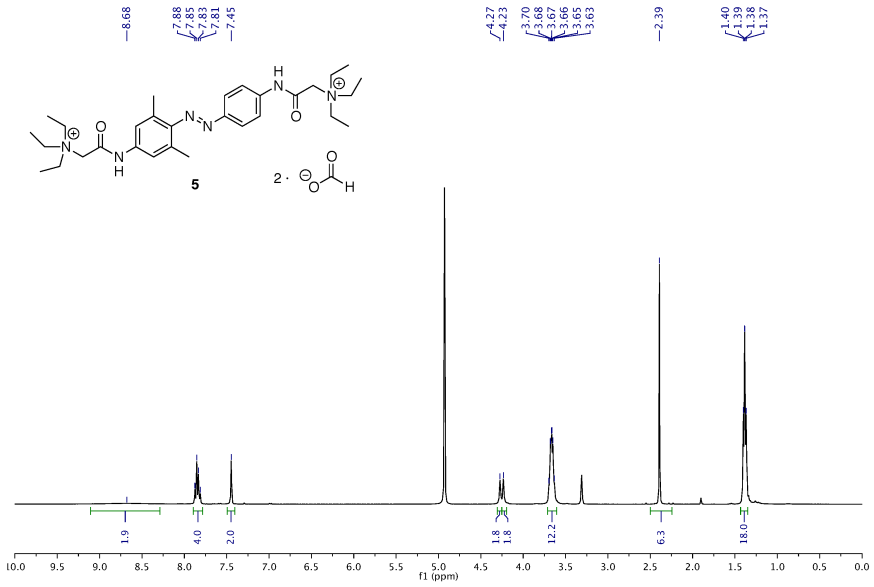
152.2
143.1
142.3
118.6
114.9
111.4
55.8
52.9
45.5
8.0
4
2 ·
f1 (ppm)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7.88
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7.81
7.45
4.27
4.23
3.70
3.68
3.67
3.66
3.65
3.63
2.39
1.40
1.39
1.38
1.37
5
2 ·
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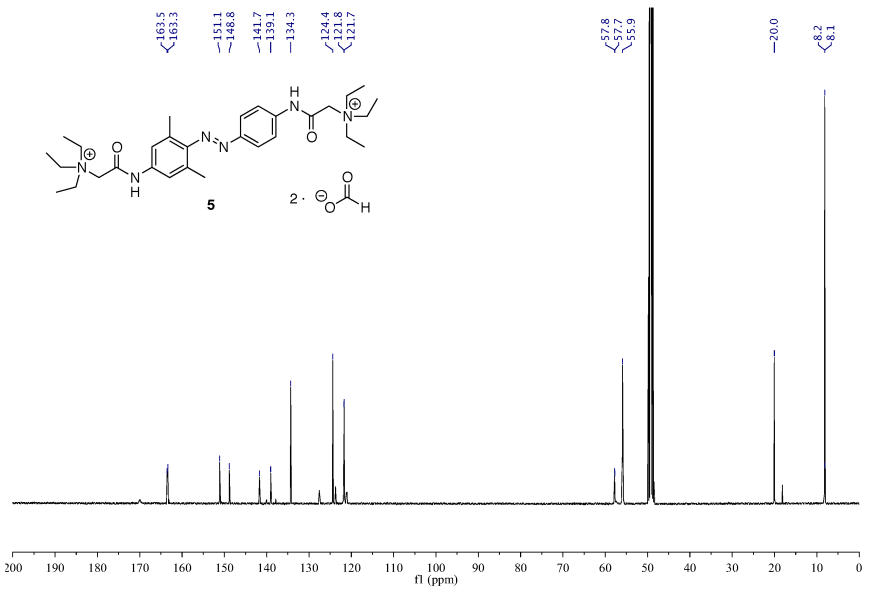
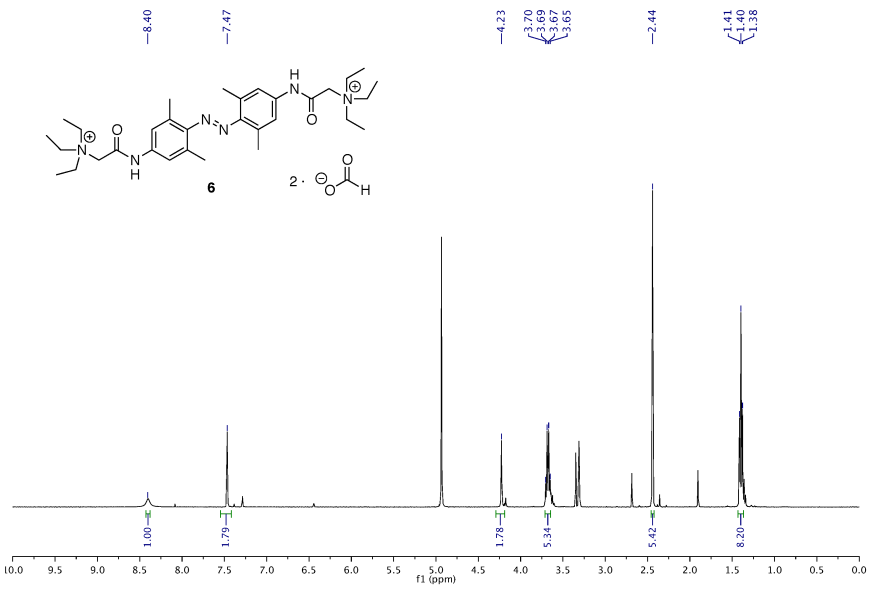
2.0
1.8
1.8
12.2
6.3
18.0
f1 (ppm)


163.5
163.3
151.1
148.8
141.7
139.1
134.3
124.4
121.8
121.7
57.8
57.7
55.9
20.0
8.2
8.1
5
2 ·
f1 (ppm)


8.40
7.47
4.23
3.70
3.69
3.67
3.65
2.44
1.41
1.40
1.38
6
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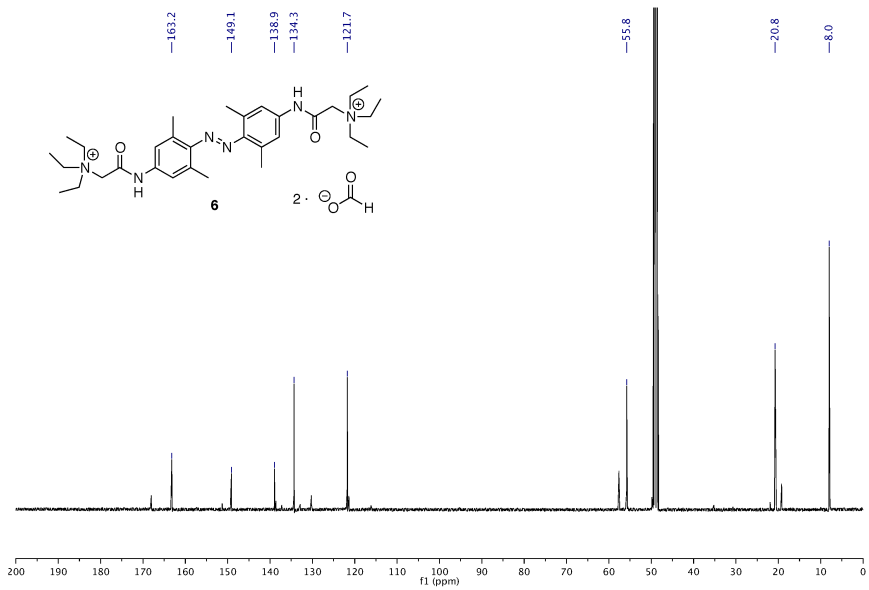
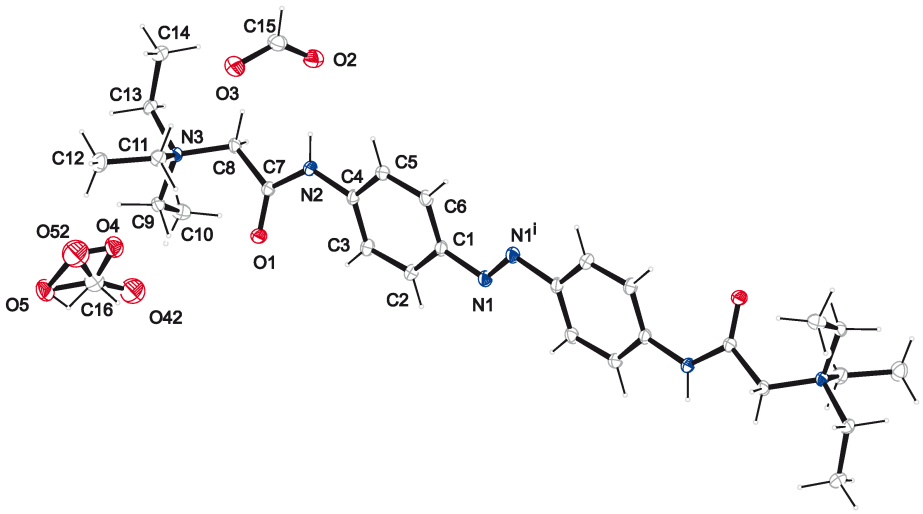
1.78
5.34
5.42
8.20
f1 (ppm)



Crystal structures

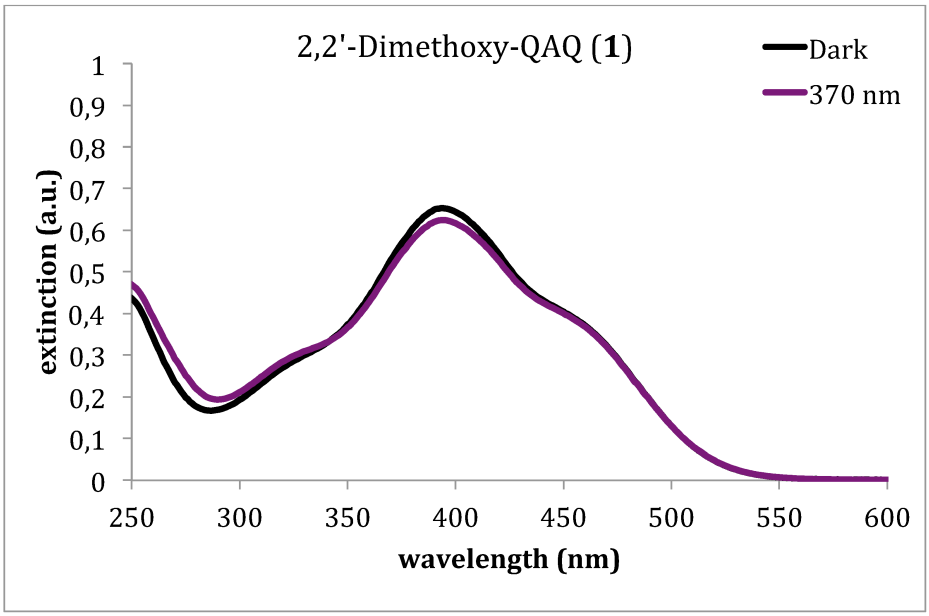
Note: Crystallographic data for QAQ has been deposited at the Cambridge Crystallographic Data Centre (CCDC 873491).

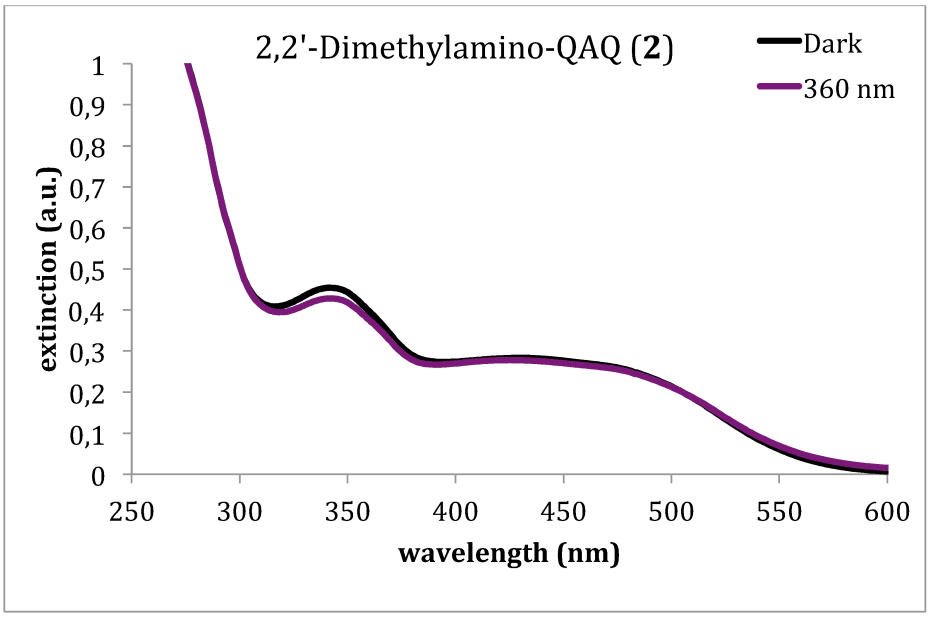
X-Ray structure of QAQ:



UV/VIS spectra of compounds 1-6

All UV/VIS spectra of compounds **1-6** were recorded in PBS solution. Compound **1** was additional recorded in DMSO.


2,2'-Dimethoxy-QAQ (1)
Dark
370 nm
extinction (a.u.)
1
0,9
0,8
0,7
0,6
0,5
0,4
0,3
0,2
0,1
0
250
300
350
400
450
500
550
600
wavelength (nm)


2,2'-Dimethylamino-QAQ (2)
Dark
360 nm
extinction (a.u.)
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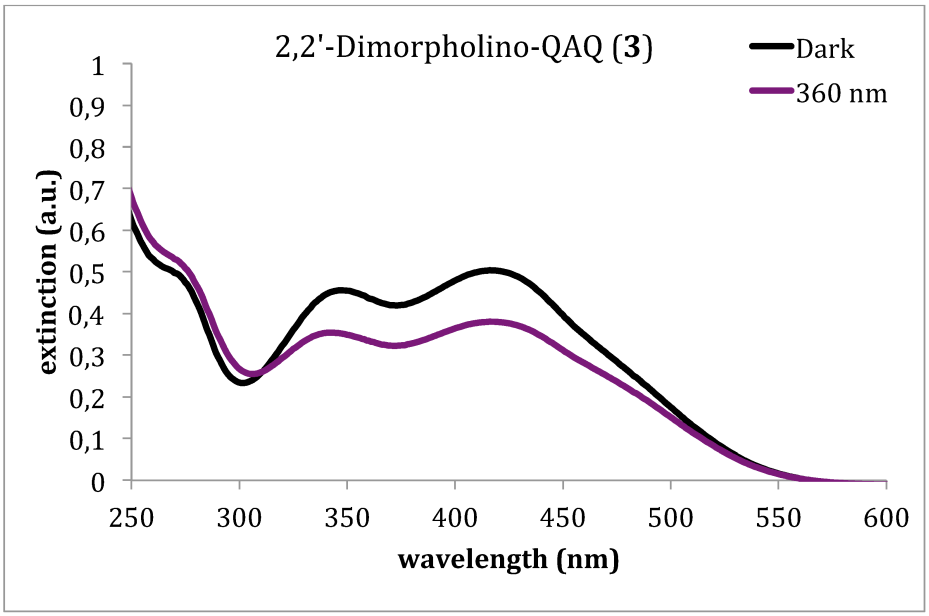
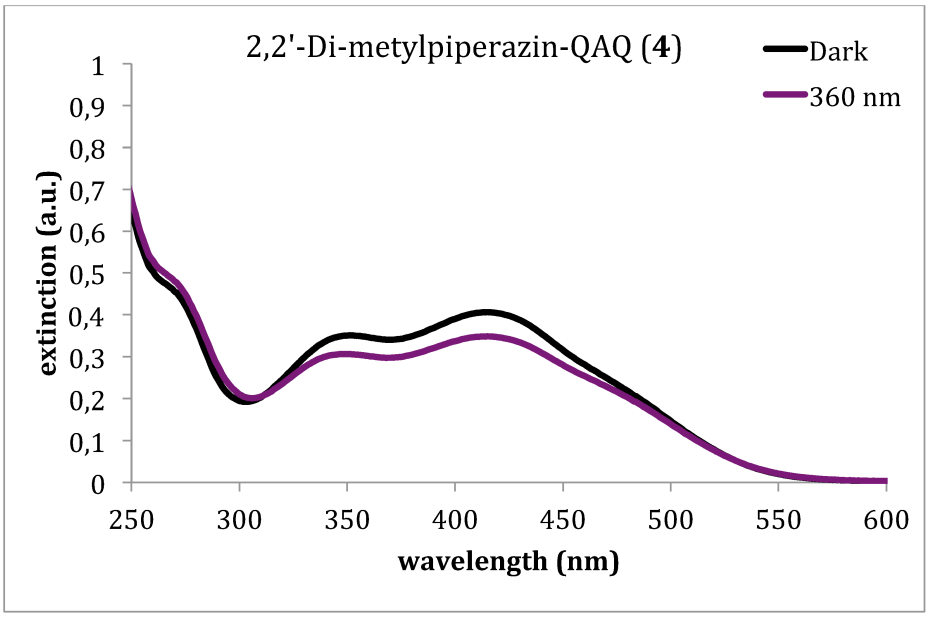
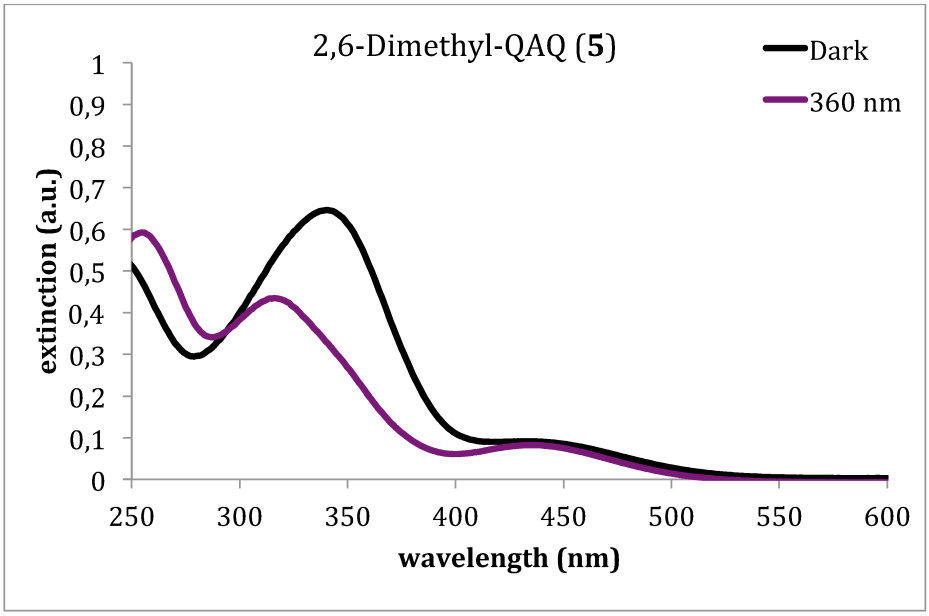
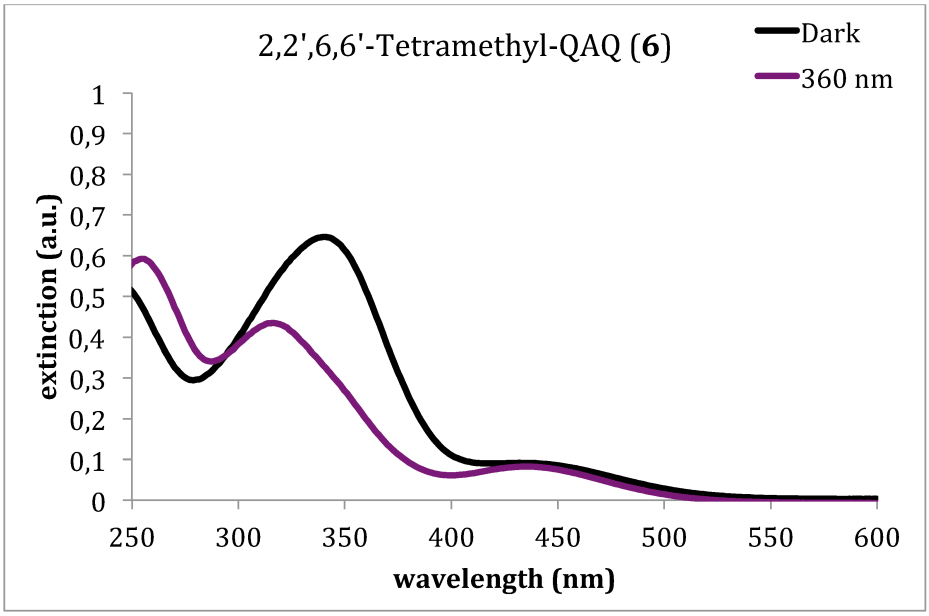
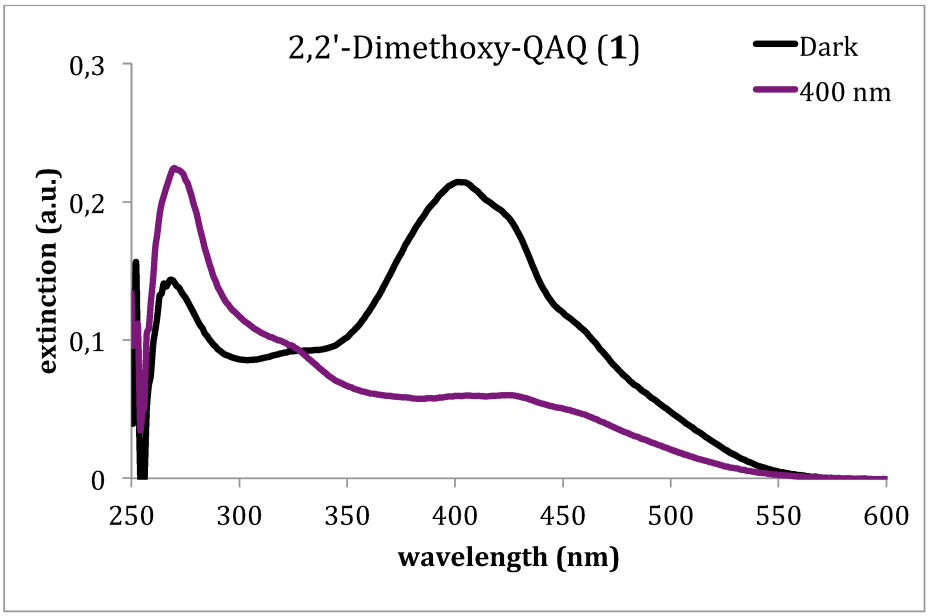
0,9
0,8
0,7
0,6
0,5
0,4
0,3
0,2
0,1
0
250
300
350
400
450
500
550
600
wavelength (nm)


2,2'-Dimorpholino-QAQ (3)
Dark
360 nm
extinction (a.u.)
1
0,9
0,8
0,7
0,6
0,5
0,4
0,3
0,2
0,1
0
250
300
350
400
450
500
550
600
wavelength (nm)


2,2'-Di-metylpiperazin-QAQ (4)
Dark
360 nm
extinction (a.u.)
1
0,9
0,8
0,7
0,6
0,5
0,4
0,3
0,2
0,1
0
250
300
350
400
450
500
550
600
wavelength (nm)

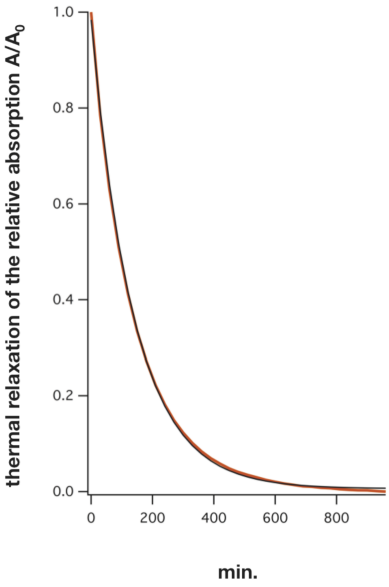

2,6-Dimethyl-QAQ (5)
Dark
360 nm
extinction (a.u.)
1
0,9
0,8
0,7
0,6
0,5
0,4
0,3
0,2
0,1
0
250
300
350
400
450
500
550
600
wavelength (nm)


2,2',6,6'-Tetramethyl-QAQ (6)
Dark
360 nm
extinction (a.u.)
1
0,9
0,8
0,7
0,6
0,5
0,4
0,3
0,2
0,1
0
250
300
350
400
450
500
550
600
wavelength (nm)



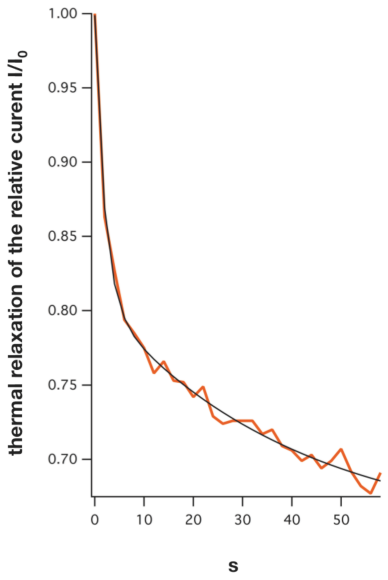
To determine the thermal relaxation of compound **1**, the spectrum above was recorded in DMSO.

Thermal relaxation of 2,6-dimethoxy-QAQ 1 in DMSO



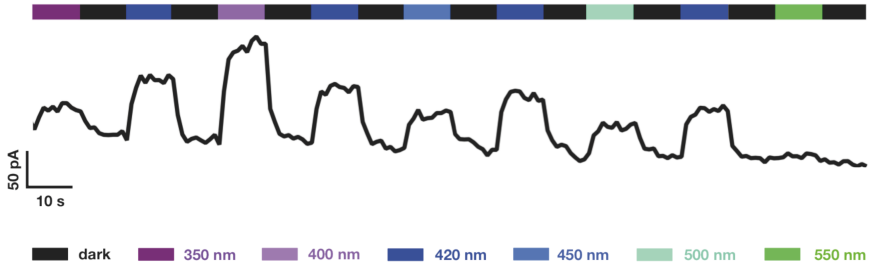
Thermal relaxation of 2,6-dimethoxy-QAQ **1**, measured by an UV-Visible Spectrophotometer in DMSO. Measurement was performed after irradiation with 400 nm. The relative absorption A/A_0 at 403 nm light, is plotted against time. Orange trace represents the recorded absorption. Black trace indicates the course of the fitted exponential function:
$y_0 + A \exp(-x/\tau)$, $y_0 = 0.006 \pm 0.002$, $A = 0.978 \pm 0.004$, $\tau = 4.61 \pm 0.04$ min. Statistics on coefficient values are presented as ± one standard deviation.

Thermal relaxation of 2,6-dimethoxy-QAQ recorded on Shaker-IR channel



Thermal relaxation of 2,6-dimethoxy-QAQ **1** recorded in voltage clamp mode, in the dark. Measurement was performed after irradiation with 420 nm. The relative Shaker-IR current I/I_0 (n = 4 cycles) is plotted against time. Orange trace represents the average of four cycles. Black trace indicates the course of the fitted biexponential function: $y_0 + A_1 \exp(-x/\tau_1) + A_2 \exp(-x/\tau_2)$, $y_0 = 0.649 \pm 0.02$, $A_1 = 0.190 \pm 0.010$, $A_2 = 0.160 \pm 0.014$, $\tau_1 = 1.948 \pm 0.226$ s, $\tau_2 = 39.454 \pm 10.8$ s. Statistics on coefficient values are presented as ± one standard deviation.

Action spectrum of 2,6-dimethoxy-QAQ recorded on Shaker-IR



Action spectrum of 2,6-dimethoxy-QAQ (**1**) recorded on Shaker-IR channels. The protocol applied depolarized the cell from - 70 mV to + 40 mV, for 250 ms at 1 Hz. After the first 250 ms of the protocol, depolarization was initiated. The voltage clamp measurements were accompanied with changing illumination, after 10 protocol cycles. Irradiation was varied between 350 nm - 550 nm light. The maximum induced current unblock is detected at 400 nm light. Wavelength higher than 500 nm light, did not unblock Shaker-IR channels. Power output of the Polychrom 5000 has not been considered.[7]

[7] Stawski, P.; Sumser, M.; Trauner, D. *Angew. Chem . Int. Ed.* **2012**, *DOI*: 10.1002/anie.201109265

Abbreviations

°C	degree Celsius
δ	NMR chemical shift in ppm
µ	micro
Ac	Acetyl
aq.	aqueous
Boc	*tert*-Butyloxycarbonyl
conc.	concentrated
DMAP	4-(dimethylamino)pyridine
DMF	*N,N*-dimethylformamide
DMSO	dimethyl sulfoxide
equiv	equivalent(s)
EI	electron impact
ESI	electron spray ionization
g	gram
h	hour(s)
HRMS	high resolution mass spectroscopy
Hz	Hertz
IR	infrared
J	coupling constant
M	mol/L
min	minute(s)
mL	milliliter
mmol	millimole
M.p.	melting point
NMR	nuclear magnetic resonance
ppm	parts per million
R_f	retention factor
rt	room temperature
sat.	saturated
THF	tetrahydrofuran
TLC	thin layer chromatography